

**Ss Cyril and Methodius University, Faculty of Natural Sciences and
Mathematics, Institute of Chemistry,
Skopje, Republic of Macedonia**

**Goce Delčev University, Faculty of Agriculture,
Štip, Republic of Macedonia**

17th International Symposium and Summer School on Bioanalysis

BOOK OF ABSTRACTS



2–8 July 2017

Congress Centre, Ohrid, Republic of Macedonia

Organizers and sponsors:

**Ss Cyril and Methodius University,
Skopje, Republic of Macedonia**

**Goce Delčev University,
Štip, Republic of Macedonia**

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WELCOME

Dear Colleagues and partners in the CEEPUS network,

It is my pleasure and honor to welcome you and your co-workers to the 17th International Symposium and Summer School on Bionalysis (17th ISSSB) organized in Ohrid, Republic Macedonia, from 2 to 8 July 2017.

The event is organized in the framework of CEEPUS CIII-RO-0010-11-1617 network. The aim of the Symposium and Summer School is to enable students and young researchers to learn and share knowledge, information and ideas about the current progress in the analytical techniques.

The symposium focuses on the recent achievements in the mainstream fields of application of analytical techniques and bioanalytical methods in chemical and pharmaceutical research, and related topics.

The scientific program includes plenary lectures, oral and poster presentations. Special attention will be given to the young researchers with sessions of podium poster communications.

I wish you a pleasant and memorable stay in Ohrid.

Trajče Stafilev

Symposium Chair, 17th ISSSB

17th International Symposium and Summer School on Bioanalysis

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Zorica Lelova, Faculty of Agriculture, Goce Delčev University, Štip, R. Macedonia

SCIENTIFIC PROGRAM

SUNDAY, 02.07.2017

15:00–19:00	Registration of participants, Congress Center, Ohrid
19:00–00:00	Welcome party, Congress Center, Ohrid

MONDAY, 03.07.2017

SESSION 1

Chairman: Trajče Stafilov (Ss Cyril and Methodius University, Skopje)

OPENING CEREMONY		
9:00–9:30	OC	Rector of Ss Cyril and Methodius University; Rector of Goce Delčev University; Ministry of Education and Science of the Republic of Macedonia; Dean of the Faculty of Natural Sciences and Mathematics, Dean of the Faculty of Agriculture, CEEPUS network coordinator
9:30–10:00	PL-01	NOVEL CE-MS METHOD TO PROFILE THE TOXIC PHOSPHOGLYCOLIPID PART OF BACTERIAL ENDOTOXINS <u>Ferenc Kilár, Anikó Kilár, Szandra Péter, Viktor Sándor, Ágnes Dörnyei, Béla Kocsis</u>
10:00–10:20	O-01	ENHANCED BIOAVAILABILITY OF DRUGS BY NANOFIBERS <u>Gabriella Donáth-Nagy, Emese Sipos, Zoltán István Szabó, Emőke Rédei, Robert Vincze, Mónika Zsombori</u>
10:20–10:40	O-02	MICROCHIP ELECTROPHORESIS IN BIOANALYSIS <u>Marián Masár, Jasna Hradski, Peter Troška, Róbert Bodor</u>
10:40–11:10	CB	Coffee break

SESSION 2

Chairman: Marián Masár (Comenius University in Bratislava)

11:10–11:40	PL-02	COMPLEXATION PROPERTIES OF AROYLHYDRAZONES <u>Nives Galić</u>
11:40–12:10	PL-03	SURFACE CHARACTERISTICS OF PDMS AND THEIR EFFECTS ON ELECTROPHORETIC SEPARATIONS <u>Attila Gaspar</u>
12:10–12:30	O-03	MICROCHIP ELECTROPHORESIS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS <u>Peter Troška, Lucia Chropeňová, Marián Masár</u>
12:30–12:50	O-04	SWEEPING OF CHARGED ANALYTES BY NEUTRAL CYCLODEXTRIN IN CAPILLARY ELECTROPHORESIS <u>Milan Boublík, Martina Riesová, Pavel Dubský, Bohuslav Gaš</u>
13:00–14:00	L	Lunch

SESSION 3		
Chairman: Ferenc Kilár (University of Pécs)		
14:00–14:30	PL-04	ADAPTING NATURAL ENZYMES TO UN-NATURAL SUBSTRATES Florin Dan Irimie, Csaba Paizs, Monica Ioana Tosa
14:30–15:00	PL-05	BRAIN CHEMISTRY PROTEOMIC AND MRI/MRS ANALYSIS TOOLS <u>Kiro Stojanoski</u> , Milena S. Kolevska, Vladimir Rendevski, Vasko Aleksovski
15:00–15:20	O-05	1H MRS METABOLITE QUANTITATION METHODS IN BRAIN CHEMISTRY <u>Milena Spasovska Kolevska</u> , Vasko Aleksovski, Vladimir Rendevski, Kiro Stojanoski
15:20–15:40	O-06	ASSESSMENT OF CHANGES IN FREEZE-DRIED PROTEIN PHARMACEUTICALS <u>Katarina Smilkov</u> , Darinka Gjorgieva Ackova, Emilija Janevik-Ivanovska, Trajče Stafilov, Zorica Arsova-Sarafinovska, Petre Makreski, Icko Gjorgoski
15:40–16:10	CB+PS	Coffee break + Poster session
16:10–17:20	PPP-01	Podium poster presentation Chairwoman: Andrea Molnar and Chairman: Leon Stojanov
19:00–21:00	D	Dinner

THUESDAY, 04.07.2017		
9:00–10:30		Visit to Hydrobiological Institute – Ohrid
10:30–14:00		Ohrid city tour
14:30–15:30	L	Lunch
SESSION 4		
Chairman: Irimie Florin Dan (Babes Bolyai University of Cluj Napoca)		
15:30–16:00	PL-06	NEUROTENSIN ANALOGUES: POTENTIAL TOOLS FOR THERAPY AND IMAGING <u>Tamara Pajpanova</u>
16:00–16:30	PL-07	POLYMERIC NANOPARTICLES WITH AGRICULTURAL AND BIOMEDICAL APPLICATIONS <u>Ede Bodoki</u>
16:30–16:40	O-07	DEGRADATION PROFILE OF ZEIN NANOPARTICLES AS TARGETED DELIVERY SYSTEMS <u>Kacsó Tímea</u> , Ioan O. Neaga, Arnold Erincz, Carlos E. Astete, Cristina M. Sabliov, Radu Oprean, Ede Bodoki
16:40–17:00	O-08	CHARACTERIZATION OF A POLY(DIMETHYLSILOXANE) MICROFLUIDIC CHIP CONTAINING IMMOBILIZED TRYPSIN FOR RAPID PROTEIN DIGESTION

		<u>Adam Kecskemeti</u> , Cynthia Nora Nagy, Attila Gaspar
17:00–17:20	O-09	CHEMICAL CHARACTERIZATION OF STATIONARY PHASES FOR FAST LIQUID CHROMATOGRAPHY <u>Dóra Zelenyánszki</u> , Nándor Lambert, Nobuo Tanaka, Attila Felinger
17:20–20:00		Swimming

WEDNESDAY, 05.07.2017

SESSION 6

Chairwoman: Marina Stefova (Ss Cyril and Methodius University, Skopje)

9:30–10:00	PL-08	PHENOLIC, VOLATILE AND ELEMENTAL COMPOSITION OF MACEDONIAN WINES <u>Violeta Ivanova-Petropulos</u> , Trajče Stafilov, Marina Stefova, Ernst Lankmayr, Ferenc Kilar
10:00–10:20	O-10	SIMPLE AND RAPID DETERMINATION OF BIOGENIC AMINES IN WINE BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY (UPLC TQ/MS) <u>Krste Tašev</u> , Violeta Ivanova-Petropulos, Marina Stefova
10:20–10:40	O-11	MALDI-TOF CHARACTERISATION OF BOTANICAL EXTRACTS FOR ENOLOGICAL APPLICATIONS <u>Arianna Ricci</u> , Giuseppina Paola Parpinello, A. Pizzi, Andrea Versari
10:40–11:00	CB	Coffee break

SESSION 7

Chairwoman: Violeta Ivanova-Petropulos (Goce Delčev University, Štip)

11:00–11:30	PL-09	MEASURING POLYMERIC PIGMENTS AND TANNINS USING SELECTED ANALYTICAL METHODS <u>Andrea Versari</u> , Arianna Ricci, Giuseppina Paola Parpinello
11:30–11:50	O-12	INFRARED SPECTROSCOPY FOR THE PREDICTION OF PHENOLICS IN WINE <u>Giuseppina Paola Parpinello</u> , Arianna Ricci, Andrea Versari
11:50–12:10	O-13	<i>IN VITRO</i> PROFILING OF MICROBIAL METABOLISM OF PHENOLIC ACIDS USING LC-MS/MS <u>Malgorzata Gwiazdon</u> , Joanna Gasik, Dorota Korsak, Magdalena Biesaga
12:10–12:30	O-14	OPTIMIZATION OF SAMPLE PRETREATMENT PHASES IN GC-MS ANALYSIS OF FATTY ACIDS Katalin Nagy, <u>Ioana Tiuca</u> , Radu Oprean
12:30–14:00	L	Lunch

SESSION 8		
Chairwoman: Margit Cichna-Markl (University of Vienna)		
14:00–14:30	PL-10	STABILITY OF POLYPHENOLS DURING EXTRACTION METHODS <u>Magdalena Biesaga</u>
14:30–14:50	O-15	EXTRACTS OF FOREST HERBS AND FRUITS – THE ANTIOXIDANT ACTIVITY AND THE CONTENT OF BIOPHENOLS <u>Paulina Drózdź</u> , Krystyna Pyrzyńska
14:50–15:10	O-16	DETERMINATION OF THE POLYPHENOLIC PROFILE OF SELECTED APPLE CULTIVARS FROM MACEDONIA WITH LC-MS ⁿ <u>Ana Petkovska</u> , Jasmina Petreska Stanoeva, Marina Stefova
15:10–15:30	O-17	COMPARISON OF DIFFERENT TYPE REVERSED PHASE STATIONARY PHASES BY MANUAL AND AUTOMATIZED COLUMN-REVERSAL METHOD Adrienn Mester, Dóra Zekenýánszki, Attila Felinger
15:30:16:00	CB+PS	Coffee break + Poster session
16:00-17:00	PPP-02	Podium poster presentation Chairwoman: Jasna Hradski and Chairman: Darko Kontrec
17:00–20:00		Swimming
20:00–00:00	CM	Dinner/Coordination meeting

THURSDAY, 06.07.2017		
SESSION 9		
Chairwoman: Magdalena Biesaga (University of Warsaw)		
9:00–09:30	PL-11	IDENTIFICATION OF BERRY SPECIES AND CULTIVARS BY DNA BARCODING Iva Nikolikj, Milena Stojkovska, Jasmina Petreska Stanoeva, Marina Stefova, <u>Margit Cichna-Markl</u>
09:30–09:50	O-18	CHARACTERIZATION OF LACTIC ACID PRODUCTION USING <i>LACTOBACILLUS CASEI</i> B-26 STRAIN IN DIFFERENT GROWTH CONDITIONS <u>Csilla Albert</u> , Zsolt Bodor, Hunor Bartos, Gyöngyvér Mara
09:50–10:10	O-19	POPPY SEED TEA, A POSSIBLE SOURCE OF MORPHINE ADDICTION OR OVERDOSE <u>Croitoru Mircea Dumitru</u> , Fülöp Ibolya, Fogarasi Erysébet, Irímia-Constrantin Maria-Raluca
10:10–10:30	O-20	ISATIS TINCTORIA OR INDIGOFERA TINCTORIA? <u>Monika Ganeczko</u> , Bartłomiej Witkowski, Magdalena Biesaga, Marcin Grzybowski, Magdalena Woźniak, Tomasz Gierczak

10:30–10:50	O-21	STUDY OF CYTOTOXIC EFFECT OF NOVEL AVPI-RGD HYBRID PEPTIDES <u>Maya Georgieva</u> , R. Detcheva, Tamara Pajpanova
11:30–20:00		Boat trip to Monastery Saint Naum of Ohrid and Ohrid springs
20:00–00:00	D	Dinner

FRIDAY, 07.07.2017		
SESSION 10		
Chairwoman: Tamara Pajpanova (Bulgarian Academy of Sciences)		
9:30–10:00	PL-12	GEOCHEMICAL ATLAS OF THE REPUBLIC OF MACEDONIA <u>Trajče Stafilov</u> , Robert Šajn
10:00–10:30	P-13	INDUCTIVELY COUPLED PLASMA SPECTROMETRY FOR THE ANALYSIS OF ENGINEERED IRON NANOPARTICLES <u>Sanda Rončević</u> , Ivan Nemet
10:30–10:50	O-22	EFFICACY OF A BIOMONITORING (MOSS) TECHNIQUE FOR DETERMINING HEAVY METALS DEPOSITION TRENDS. CASE STUDY BREGALNICA RIVER BASIN, REPUBLIC OF MACEDONIA <u>Biljana Balabanova</u> , <u>Trajče Stafilov</u> , Robert Šajn
10:50–11:20	CB	Coffee break
SESSION 11		
Chairwoman: Nives Galić (University of Zagreb)		
11:20–11:40	O-23	THE EFFECT OF PGP BACTERIAL INOCULATION ON THE ACCUMULATION OF HEAVY METALS IN CROP PLANTS <u>Gyöngyvér Mara</u> , Éva Boglárka Vincze, Annamária Becze, Csilla Albert
11:40–12:10	O-24	THE INFLUENCE OF UV-VIS IRRADIATION AND OXIDATION ON ARSENIC AND CHROMIUM SPECIATION IN WATER <u>Ewa Biaduń</u> , Beata Krasnodębska-Ostrega, Krzysztof Miecznikowski
12:10–12:30	O-25	SORPTION OF Sc(III) ON SOLID CARBON NANOMATERIALS <u>Mateusz L. Pegier</u> , Krzysztof Kilian, Krystyna Pyrzyńska
13:00–14:00	L	Lunch
SESSION 12		
Chairwoman: Livia Uncu (State University of Medicine and Pharmacy "Nicolae Testemitanu")		
14:00–14:30	PL-14	CHEMISTRY, ENVIRONMENTAL FATE AND TRANSFORMATION OF HEXACHLOROCYCLOHEXANES INTO ECOLOGICALLY ACCEPTABLE DERIVATIVES <u>Jane Bogdanov</u>

14:30–14:50	O-26	APPLICATION OF 2-D ELECTROPHORESIS AND MALDI TOF MS ANALYSIS FOR THE QUALITY STUDY OF BIOLOGICS <u>Dashnor Nebija</u> , Christian Noe, Christina Mladenovska and Bodo Lachmann
14:50–16:10	O-27	STUDY OF THE OXIDATIVE METABOLIZATION PATTERN OF BETA-BLOCKERS <u>Ruxandra Chira</u> , Ede Bodoki, Viktor Sándor, Ferenc Kílár, Radu Oprean
16:10–17:00	CB+PS	Coffee break + Poster session
17:00–18:10	PPP-03	Podium poster presentation Chairwoman: Cynthia Nora Nagy and Chairman: Georg Clemens Pretsch
18:10–20:00	CB-02	Swimming
20:00–21:00	D	Dinner

SATURDAY, 08.07.2016

SESSION 13

Chairwoman: Gabriella Donáth-Nagy (University of Medicine and Pharmacy of Tg. Mureş)

9:30–10:00	PL-15	THE USE OF HPLC METHOD IN ANALYSIS OF MULTICOMPONENT DRUGS <u>Livia Uncu</u>
10:00–10:20	O-28	THE OPTIMIZATION OF THE BIOAVAILABILITY OF THE PROPYLTHIOCIOTHIAZOLE <u>Andrei Uncu</u> , Ana Podgornîi, Anastasia Smetanscaia, Fliur Macaev, Vladimir Valica, Eva Tesarova
10:20–10:40	O-29	NOVEL APPROACH FOR DNA METHYLATION ANALYSIS IN REGULATORY REGIONS OF MGMT IN GLIOBLASTOMA <u>Katja Zappe</u> , Ru Wang Qiu, Andreas Böhm, Sabine Spiegl-Kreinecker, Margit Cichna-Markl
10:40–11:00	O-30	DETERMINATION OF MIDAZOLAM AND ITS MAIN METABOLITES IN URINE AND SERUM SAMPLES BY LC-UV AND LC-MS <u>Mariusz Procak</u> , Sebastian Rojek, Michael Hiesmayr, Margit Cichna-Markl
11:00–11:30	CB	Coffee break

SESSION 14

Chairman: Attila Gaspar (University of Debrecen)

11:30–12:00	PL-16	QUALITY ASSESSMENT OF THE DOCTORAL SCHOOLS IN THE FIELD OF BIOANALYSIS <u>Radu Oprean</u>
12:00–12:20	O-31	GUEST – HOST INTERACTION STUDIES BETWEEN PROPRANOLOL AND B-CYCLODEXRIN AT SOLID/LIQUID INTERFACE <u>Ioan – Adrian Stoian</u> , Bogdan-Cezar Iacob, Ede Bodoki, Radu Oprean
12:20–12:40	O-32	FOOD ADULTERATION – A TETRAPLEX REAL-TIME PCR ASSAY FOR THE SIMULTANEOUS DETERMINATION OF FOUR FREQUENTLY

		CONSUMED GAME ANIMALS <u>Maria Kaltenbrunner</u> , Rupert Hochegger, Margit Cichna-Markl
13:00-14:00	L	Lunch
14:00-14:30		Closing remarks

POSTER PRESENTATIONS

PPP-01	Podium poster presentation-01
P-01	THE USE OF ELECTROLYTE SYSTEMS COMPATIBLE TO MASS SPECTROMETRIC DETERMINATION FOR CAPILLARY ELECTROPHORESIS <u>Andrea Molnar</u> , Adam Kecskemeti, Attila Gaspar
P-02	TRYPTIC DIGESTION OF TEAR SAMPLES USING A MICROFLUIDIC ENZYME REACTOR <u>Cynthia Nora Nagy</u> , Adam Kecskemeti, Attila Gaspar
P-03	ANALYTICAL INSTRUMENTS FOR BIOPHARMACEUTICAL CHARACTERIZATION <u>Darinka Gjorgieva Ackova</u> , Katarina Smilkov, Emilija Janevik-Ivanovska, Trajče Stafilov, Zorica Arsova-Sarafinovska, Petre Makreski
P-04	EVALUATION OF THE REDOX INTERACTION OF RUTIN WITH SOME MEDICINAL SUBSTANCES BY CYCLIC VOLTAMMETRY <u>Mihail Aleksandrov</u> , Viktorija Maksimova, Rubin Gulaboski
P-05	CYTOTOXICITY ASSESSMENT OF AROYLHYDRAZONE DERIVATIVES <u>Darko Kontrec</u> , Ivan Iliev, Roumiana Detcheva, Nives Galić, <u>Tamara Paipanova</u>
P-06	SIGNIFICANCE OF THE NON-COVALENT INTERMOLECULAR INTERACTIONS IN MOLECULAR COMPLEXES OF PHARMACEUTICAL RELEVANCE <u>Aleksandar Cvetkovski</u>
P-07	USE OF MICROBORE SIZE-EXCLUSION CHROMATOGRAPHY FOR THE CHARACTERIZATION OF MACROMOLECULAR SAMPLES <u>Erik Beňo</u> , Róbert Góra, Milan Hutta
P-08	PRELIMINARY RESULTS OF COUPLING MICROCHIP ELECTROPHORESIS WITH ION MOBILITY SPECTROMETRY <u>Jasna Hradski</u> , Michaela Nováková, Martin Sabo, Štefan Matejčík, Marián Masár
P-09	HYBRID PH-SENSITIVE NANOPARTICLES AS PLATFORMS FOR DELIVERY OF CURCUMIN <u>Elena Drakalska</u> , Denitsa Momekova, Nikolay Lambov, Stanislav Rangelov, Bistra Angelovska
P-10	FREEZE DRIED KIT FORMULATION OF TRASTUZUMAB IMMUNOCONJUGATES <u>Marija Sterjova</u> , Emilija Janevik-Ivanovska, Paulina Apostolova, Predrag Dzodic
P-11	DNA METHYLATION ANALYSIS OF HIPPOCAMPAL TISSUE FROM AGED RATS <u>Georg Clemens Pretsch</u> , Margit Cichna-Markl
P-12	IN VITRO EVALUATION OF FOOD-DICLOFENAC INTERACTIONS <u>Ibolya Fülöp</u> , Mircea Dumitru Croitoru, Andrea Vajda, Irén Jakab, Erzsébet Fogarasi

PPP-02	Podium poster presentation-02
P-13	OPTIMIZATION AND VALIDATION OF CAPILLARY ELECTROPHORESIS METHOD FOR SMALL-ANIONS MEASUREMENT IN RED WINES <u>Zorica Lelova</u> , Violeta Ivanova-Petropulos, Marián Masár, Klemen Lisjak, Róbert Bodor
P-14	DEVELOPMENT OF HPLC/DAD/MS ⁿ METHOD FOR AUTHENTICATION OF FRUIT PRODUCTS <u>Milena Stojkovska</u> , Jasmina Petreska Stanoeva, Marina Stefova, Margit Cichna-Markl
P-15	PINDOLOL – ENANTIOSEPARATION AND VALIDATION OF THE METHOD <u>Martin Ansorge</u> , Lívie Kanizsová, Iva Zusková
P-16	RRLC-UV CHROMATOGRAPHIC METHOD USED FOR DETERMINATION OF CHLOROGENIC ACID IN GREEN COFFEE Lenche Velkoska-Markovska, <u>Mirjana S. Jankulovska</u> , Biljana Petanovska-Ilievska, Kristijan Hristovski
P-17	COUPLING CHROMATOGRAPHIC MICROFLUIDIC DEVICES WITH GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETER <u>Arpad Kiss</u> , Attila Gaspar
P-18	APPLICATION OF Q-ICP-MS FOR SENSITIVE DETERMINATION OF LEAD ISOTOPE RATIOS IN VARIOUS ORGANICALLY BASED MATRIXES <u>Biljana Balabanova</u> , Violeta Ivanova-Petropulos, Blazo Boev
P-19	ASSESSING THE BIOAVAILABILITY AND TRANSLOCATION EFFICIENCY OF MINERAL ELEMENTS IN <i>Lycium barbarum</i> SPECIES FROM R. MACEDONIA AND R. CHINA <u>Biljana Balabanova</u> , Violeta Ivanova-Petropulos, Tiberiu Frentiu, Michaela Ponta, Eniko Covaci, Marin Senila
P-20	CHEMICAL PROPERTIES OF CERTAIN VARIETIES OF TOBACCO <u>Romina Kabranova</u> , Zlatko Arsov, Zoran Dimov, Mirjana S. Jankulovska
P-21	PHOSPHORUS AND POTASSIUM AVAILABILITY IN SOILS USED FOR TOBACCO CULTIVATION IN THE REPUBLIC OF MACEDONIA <u>Biljana Jordanoska</u> , Valentina Pelivanoska, Trajče Stafilov
P-22	UV SPECTROSCOPY METHOD USED IN DETERMINATION OF DISSOCIATION CONSTANT OF SOME p-NITRO-p-SUBSTITUTED BENZOILHYDRAZONES <u>Mirjana S. Jankulovska</u> , Vesna Dimova, Ilinka Spirevska
P-23	STUDY OF THE RELATIONSHIPS BETWEEN THE STRUCTURE AND BIOLOGICAL ACTIVITY OF SOME SUBSTITUTED AROMATIC HYDRAZONES Vesna Dimova, <u>Mirjana Jankulovska</u>
P-24	SYNTHESIS OF NOVEL AMINOMETHYL DERIVATES OF QUINOLONES <u>Emilija Gjorgieva</u> , Emil Popovski

PPP-03	Podium poster presentation-03
P-25	REPARATION OF SILVER NANOPARTICLES USING ASCORBIC ACID AND GLUTHATHIONE AS REDUCTIVE REDOX AGENTS <u>Leon Stojanov</u> , Valentin Mirceski
P-26	RELATION BETWEEN STRUCTURE AND ACTIVITY OF ANTISEPTICS AND DISINFECTANTS THAT ARE USED IN CLINICS Biljana Gorgeska, <u>Andonela Janeva</u> , Ivana Iceva, Dino Karpicarov, Antonela Velkova, Viktorija Krzovska, Ana Dimitrova, Natali Delipetrova
P-27	FAST DETERMINATION OF GLYPHOSATE IN BEER BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY <u>Krste Tašev</u> , Valentina Pantò, Angelo Faberi, Suzana Krstevska, Ana Stefanovska, Ljupka Kostovska
P-28	IDENTIFICATION OF PESTICIDES IN GROUNDWATER SITUATED UNDER GREENHOUSE AGRICULTURE PRODUCTION AND DROPPING IRRIGATION, USING GC/MS PULSED SPLITLESS INJECTION <u>Biljana Kovacevik</u> , Zoran Zdravkovski, Sasa Mitrev
P-29	DEVELOPMENT OF A SINGLE-DROP MICROEXTRACTION METHOD FOR GC-MS ANALYSIS OF BISPHENOL A IN WATER SOLUBLE MATRICES <u>Katarina Josifovska</u> , Zoran Zdravkovski
P-30	SOYBEAN VARIETIES AS EFFECTIVE TOOL FOR PHYTOREMEDIATION OF CADMIUM POLLUTED SOIL Ljupcho Mihajlov, <u>Biljana Balabanova</u> , Vesna Zajkova Panova, Shuhe Wei
P-31	ASSESSMENT OF ARSENIC POLLUTED GROUNDWATER IN THE STRUMICA REGION, AN INTENSIVE AGRICULTURE PRODUCTION AREA <u>Biljana Kovacevik</u> , Blazo Boev, Vesna Zajkova Panova, Sasa Mitrev
P-32	MACRO AND TRACE ELEMENTS BIOAVAILABILITY IN VEGETABLE AND HERBAL SPECIES FROM POLLUTED AND CONTROL AREAS <u>Biljana Balabanova</u> , Trajče Stafilov, Liping Fan, Meicong Wang, Yanqiu Liang, Minxiu Yan
P-33	MULTI-ELEMENT CONTENT CHARACTERIZATION OF COLD PRESS EDDIBLE OILS PRODUCED FROM TWELVE SUNFLOWER VARIETIES Ivan Donev, <u>Biljana Balabanova</u> , Sasa Mitrev
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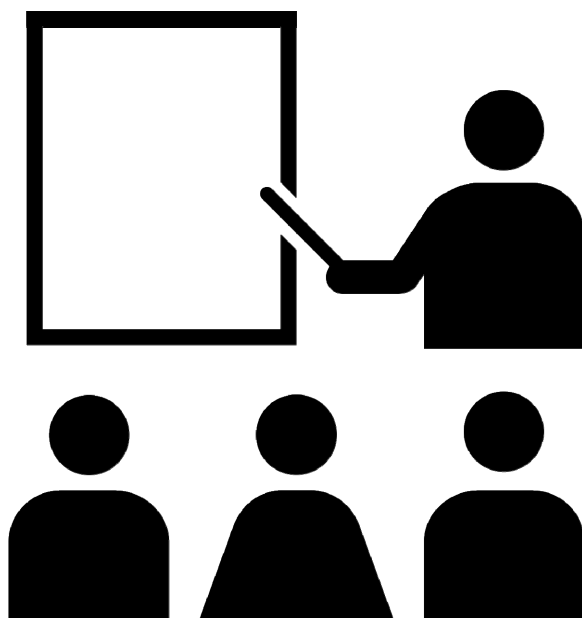
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PLENARY LECTURES



PL-01

NOVEL CE-MS METHOD TO PROFILE THE TOXIC PHOSPHOGLYCOLIPID PART OF BACTERIAL ENDOTOXINS

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Bacterial endotoxins (lipopolysaccharides, LPSs) are important initiators of sepsis, a clinical syndrome that is a leading cause of death in intensive care units. LPSs are found on the surface of Gram-negative bacteria and are anchored to the cell membrane by their phosphoglycolipid moiety (lipid-A), which is responsible for toxicity. Main challenges in the structure elucidation of these molecules arise from their amphiphilic character and extreme heterogeneity. We developed a capillary electrophoresis electrospray ionization mass spectrometry method for the identification of these structurally closely related compounds.

Electrophoretic separation by CE-ESI-ion trap MS of 20 lipid-A components related to *E. coli* O112 bacterium was accomplished [1, 2]. The migration order corresponded to the increasing charge to mass values. The net charge and size of the deprotonated lipid-A molecules depended on their phosphorylation (mono- or di-) and acylation degrees (ranging from di- to hexa-acylation). The optimized CE electrolyte fulfilled the requirements necessary for (i) dissolving the amphipatic lipid-A molecules, (ii) the electrophoretic separation and (iii) sufficient ionization during the ESI process. The selectivity of the MS could be used to distinguish between co-migrating compounds, and mass isomers (isobars).

CE-ESI-ion trap MS offers an attractive alternative for the analysis of lipid-A extracts, as it combines the high separation efficiency of CE with the possibility of mass-selective detection.

Keywords: endotoxin, lipid A, CE-ESI-MS, structure elucidation.

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PL-02

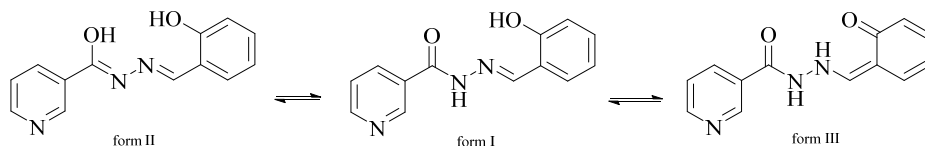
COMPLEXATION PROPERTIES OF AROYLHYDRAZONES

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Aroylhydrazones can be involved in keto-enol tautomeric interconversion (Scheme I, forms I and II). Tautomeric equilibrium can also involve aldehyde moiety if the hydroxyl group is situated in *ortho* position with respect to the C=N double bond (Scheme I, form III). As a consequence, aroylhydrazones can act as neutral, monoanionic or dianionic ONO tridentate ligands [1].



Scheme 1. Possible tautomeric forms of aroylhydrazones

Biological systems have difficulties to differentiate between Fe^{3+} and Ga^{3+} ions due to their nearly identical ionic radii, same charge, preferred coordination number and chemical behavior. However, Ga^{3+} lacks the redox activity of iron (3+/2+ redox chemistry) and is marked as “Trojan Horse” in biological systems [2]. In this lecture the coordination abilities of aromatic hydrazones derived from nicotinic acid hydrazide and differently substituted 2-hydroxybenzaldehydes towards Fe^{3+} and Ga^{3+} will be discussed. Stability constants and the stoichiometry of the corresponding M^{3+} :hydrazone complexes in MeOH/H₂O media were determined spectrophotometrically. Mass spectrometry was used for structural characterization of the complexes in solution. The solid complexes of the examined ligands with Fe^{3+} were isolated and characterized as well.

Keywords: aromatic hydrazones, iron(III), gallium(III), UV-Vis spectrometry, mass spectrometry.

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PL-03

SURFACE CHARACTERISTICS OF PDMS AND THEIR EFFECTS ON ELECTROPHORETIC SEPARATIONS

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Poly(dimethylsiloxane) (PDMS) is frequently the material of choice in analytical chemistry when fabricating hydrophobic and/or adsorptive surfaces or microfluidic devices. In general, it is low cost, is easily machined, and offers a number of other properties amenable to its use in a variety of applications. PDMS allows for the development of devices offering many advantages over non-microfluidic-based techniques including small sample volume requirements, portability, low production costs per device, speed of analysis, versatile format for integration of various detection schemes, parallel processing of samples and ability to multiplex and compatibility with other techniques.

Because microfluidic chips have a high surface-to-volume ratio, interactions between the samples and the surface become very important. It is well known that adsorption and absorption of many types of molecules occur on the surface of PDMS. The adsorption is known to cause band broadening (tailing) of components and fluctuation in the rate of electroosmotic flow (EOF) during electrophoretic separations on microchips. We demonstrated that the adsorption (either reversible or irreversible) interactions between PDMS and small molecules, pharmaceuticals and proteins can be investigated by surface plasmon resonance (SPR) in real-time and by label-free means thereby expanding the knowledge base regarding adsorption effects and surface modification of PDMS [1]. We also showed that the PDMS chips can be applied as effective enzyme reactors for digestion of proteins [2].

Keywords: poly(dimethylsiloxane), adsorption, electrophoresis, electroosmosis, microreactor, surface plasmon resonance.

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PL-04

ADAPTING NATURAL ENZYMES TO UN-NATURAL SUBSTRATES

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The evolution of each enzyme continuously adapts it to its function - to catalyze a certain chemical reaction, working with a dedicated substrate in specific conditions.

Yet enzymes' promiscuity remains a reality that can be exploited by chemists, to conduct the same reaction with different substrates, and in other (modified) conditions.

In time, techniques have been developed to accommodate the enzyme to the conditions of a chemical laboratory, or to those of a reactor, where it will transform a substrate which is different from his natural one. The presentation will provide practical examples.

Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number PN-II-PT-PCCA-2013-4-1006.

PL-05

BRAIN CHEMISTRY PROTEOMIC AND MRI/MRS ANALYSIS TOOLS

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Our main goal in this presentation is a part of a larger effort to develop methods and approaches in order to understand protein diversity of a human proteome in health and neurological disease [1]. Primarily, we will focus on the new more invasive evolving proteomic methods in brain chemistry mainly for cerebrospinal fluid (CSF) and blood analysis for study of neurological diseases. One of the methods is electrophoresis technique which is the hub of laboratory testing and a component of almost all diagnostic probes for the protein chemistry. By combining mass spectrometry with gel electrophoresis, and or immunochemistry methods with two dimensional gel electrophoresis is created coupled power tools for diagnosis. In the “single protein” approach from complex protein mixtures analysis *mass spectrometry immunoassay* (MSIA) was used. In biological specimens that are commonly used for biomarker assessment, proteins are present in a large span of concentrations. Therefore, detection and analysis of protein biomarkers and other metabolite is very complex and challenging. In this presentation an attempt was made to switch from biofluid circulatory metabolite concentration to *in situ* tissue or cell analysis. We will discuss the present status and the future of the less invasive techniques in the brain chemistry metabolite analysis such as *magnetic resonance imaging* (MRI) and *magnetic resonance spectroscopy* (MRS) [2]. Quantitative aspects and new approaches in the ¹H MRS as tool for “noninvasive brain neurobiopsy” will be explained on the some normal and neural brain diseases.

Keywords: electrophoresis, MRI/MRS analysis tools, MSIA.

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PL-06

NEUROTENSIN ANALOGUES: POTENTIAL TOOLS FOR THERAPY AND IMAGING

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Neurotensin (NT) is a tridecapeptide with the sequence pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH, which is located and produced in the gastrointestinal tract, the central nervous system, and the brain. Like other neuropeptides, neurotensin has different functions. It is a neurotransmitter and neuromodulator in the central nervous system and a local paracrine hormone in the periphery, particularly in the gastrointestinal tract.

For neurotensin three different receptors (NTS1, NTS2, and NTS3) were cloned and studied thus far. Neurotensin receptor 1 (NTS1) and neurotensin receptor 2 (NTS2) belong to the class A of G protein-coupled receptors (GPCRs), which are responsible for most of the biological effects associated with neurotensin. Receptor subtype 3 (NTS3) is part of the Vps10p family of sorting receptors.

NTS1 triggers analgesic and antipsychotic response and controls dopamine-mediated neuroleptic effects. Accordingly, NTS1 plays a role in psychiatric and neurological diseases, e.g. in the pathogenesis of Parkinson's disease or schizophrenia. Moreover, NTS1 stimulation is involved in the promotion of cancer growth.

In recent decade, there has been a great interest in the development of radiolabeled small peptides for diagnostic imaging and radionuclide therapy in nuclear oncology. Neurotensin receptor 1 (NTS1) is overexpressed on a variety of cancer entities; for example, prostate cancer, ductal pancreatic adenocarcinoma, and breast cancer. Therefore, it represents an interesting target for the diagnosis of these cancers types by positron emission tomography (PET).

Keywords: neurotensin, Parkinson's disease, imaging.

PL-07

POLYMERIC NANOPARTICLES WITH AGRICULTURAL AND BIOMEDICAL APPLICATIONS

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The advent of nanotechnology has enabled distribution and disposal of engineered nanomaterials in various areas such as medicine, food, agriculture, electronics, etc. Through smart engineering nanostructured polymeric materials become highly versatile and sophisticated tools that are able to demonstrate multiple advantages originating from their high surface area and tunable surface chemistry. One of the features of such nanoscaled materials exploited with an increasing frequency in almost every field of life sciences is the targeted, controlled and sustained delivery of various (bio)active chemicals. Two distinct application fields, in agriculture and health, employing pesticides and antioxidants entrapped in biocompatible and biodegradable organic nanocarriers (poly(lactic co-glycolic) acid and zein) will be discussed. Particularities in the formulation design, expected and recorded product efficacy as well as the bioanalytical challenges encountered throughout *in vitro* and *in vivo* experiments will also be presented. Furthermore, in order to ensure safe and sustainable implementation of such nanotechnology products, it is critical that the interaction of nanoparticles with animal, plant, soil, air, and water systems be thoroughly elucidated. Therefore, certain aspects related to their accelerated degradation in environmentally relevant conditions as well as polymeric nanoparticles-plant interaction studies simulating accidental or intentional exposure to such nanoparticles will also be pointed out.

Keywords: polymeric nanoparticles, targeted drug delivery, nanopesticides, nanoparticle-plant interactions, accelerated degradation, sustainable nanotechnology.

Acknowledgement: The Fulbright Senior Award granted by the Romanian – U.S. Fulbright Commission to Ede Bodoki, as well as the support offered by the CEEPUS network No. CIII-RO-0010-11-1617 - Teaching and Learning Bioanalysis is greatly acknowledged.

PL-08

PHENOLIC, VOLATILE AND ELEMENTAL COMPOSITION OF MACEDONIAN WINES

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Wine is a very complex mixture of a large number of compounds, including phenolics (anthocyanins, flavan-3-ols, flavonols, phenolic acids, stilbenes), volatiles (alcohols, esters, aldehydes, volatile phenols), minerals (Al, Zn, Cu, Fe, Pb), organic acids, carbohydrates, proteins, vitamins etc. All these compounds significantly influence the quality of wine, affecting the sensory perception such as flavor, aroma and colour, pH, chemical and microbiological stability of wines. Their amount in wine depends on various factors such as grape variety, environmental factors, ripening stage, vine cultivation and winemaking practices applied for wine production. The winemaking process is one among these factors that can be controlled, and mainly includes a range of modifications during fermentation and maceration, such as fermentation temperature, yeast strain, racking duration, type of lees, etc. This paper summarize the results obtained for phenolic, volatile and elemental composition of Macedonian wines, commercial as well as wines produced under different winemaking techniques, analyzed with the most sophisticated techniques such as HPLC-DAD-ESI-MS/MS, MALDI-TOF-MS, CZE-ESI/QTOF-MS, HS-GC-MS and ICP-MS [1-10]. Obtained results give us new information about the quality of wines, which is important to support the viticulture and wine strategy in Macedonia as well as to increase the competitiveness of Macedonian wines on the global market as products with specific quality characteristics, such as high content of phenolics and antioxidants, rich in color and aroma.

Keywords: phenolic compounds, volatiles, elemental composition, wine, analytical techniques.

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PL-09

MEASURING POLYMERIC PIGMENTS AND TANNINS USING SELECTED ANALYTICAL METHODS

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Anthocyanins and tannins are antioxidant molecules that play an important role to the overall quality of red wines (e.g. red color, astringency) depending on their structure and interaction, and the wine industry is seeking for improved analytical methods for managing anthocyanins and tannins in winemaking.

Our current aim is to investigate the behavior of some of the most common food grade tannins by using selected analytical methods (spectrometry, HPLC, Cyclic Voltammetry) for predicting the tannin content and looking for the main structural features (e.g. radical scavenging activity, reducing power and redox properties) which can explain the role of tannins in driving the wine aging.

Rapid spectrometry methods are critically discussed and results compared with developed HPLC analysis of polymeric pigments and tannins. Spectral data were processed to classify tannins based on their botanical origin, whereas the Cyclic Voltammetry signal at 500 mV was highly correlated with DPPH• value due to the catechol ring of flavonoids and trigalloyl moieties of gallic acid-based compounds. Practical examples of tannins application in winemaking are presented.

Keywords: tannins; polymeric pigments; quality control; antioxidant.

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PL-10

STABILITY OF POLYPHENOLS DURING EXTRACTION METHODS

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Polyphenolic compounds belong to the large group of secondary plant metabolites. They have excellent antioxidant activities against reactive oxygen species. These properties have been exploited to prevent diseases associated with oxidative damage such as coronary heart disease, stroke and cancers.

HPLC with different detectors is widely used for the determination of polyphenols in food samples. The stabilities of several flavonoids and phenolic acids in food samples during different extraction modes such as reflux heating, maceration, ultrasonic extraction (USE) and microwave-assisted extraction (MAE) are compared. The smallest decomposition was observed by heated reflux extraction procedure within 30 min in water bath. Phenolic compounds from the standard mixture seem to be stable under ultrasounds action with the mean recovery of $(90.4 \pm 7.1) \%$, but during microwave-assisted extraction the benzoic acid derivatives and aglycones of flavonoids showed lower recovery (70-80%).

In honey, apple, onion samples, it was found that the phenolic acids and the glycosides exhibited high stability to MAE and USE treatments. In the presence of a high artificial sugar matrix, flavonols were almost degraded after successive irradiation assisted extraction. Application of the USE conditions provided higher and/or similar extraction yields for phenolic acids than usually applied shaking with solvent. It also allowed shortening the time required for the whole procedure of sample preparation.

The effect of degradation of polyphenols depend on extraction mode, time of extraction, chemical structure and type of food matrix were studied. The most unstable compound (recovery below 50%) in tested condition was myricetin. The stability of tested compounds strongly depends on their structures and pH of matrix. The higher number of hydroxyl groups promotes degradation of flavonoids, whereas sugar moiety and methoxyl groups protect flavonoids from degradation during microwave and ultrasonic assisted extraction. Lower pH of samples protects the polyphenols degradation.

Keywords: polyphenol, extraction, food samples, LC-MS.

PL-11

IDENTIFICATION OF BERRY SPECIES AND CULTIVARS BY DNA BARCODING

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Epidemiological studies suggest that regular consumption of fruits reduces the risk of chronic and degenerative human diseases. In recent years, the consumption of berries and berry products has become very popular. However, studies indicate that berry products are frequently adulterated and thus do not comply with national and European Union food regulations. Most commonly, berries of higher value are replaced by less expensive berries or even by other fruit species.

Analytical methods are required to verify if berry products actually contain the berries species declared. Several studies showed that DNA barcoding has a high potential for species differentiation in food. Papers dealing with the differentiation of berry and fruit species by DNA barcoding are, however, scarce.

In DNA barcoding, distinctive regions in the DNA, so-called “DNA barcodes”, are analyzed with the aim to identify and differentiate organisms. DNA barcoding includes selection of an appropriate barcode, the amplification of the selected barcode region by the polymerase chain reaction (PCR) and analyzing the PCR products, e.g. by sequencing or high resolution melting (HRM). In order to be applicable, a DNA barcode should contain a central variable part, allowing the discrimination of the species or cultivars of interest. The variable part should be flanked by conserved regions, making it possible to use a universal primer pair for amplifying the barcode sequence in different species/cultivars.

After explaining the principle of DNA barcoding in more detail, the lecture will give an overview of DNA barcode regions commonly used for plant species identification. In addition, the lecture will present results from our project obtained with DNA barcoding by HRM analysis, e.g. on the differentiation between cranberry and lingonberry or the differentiation of pomegranate cultivars. These examples will be used to discuss the advantages, but also the limitations of this technology.

Keywords: DNA barcoding, High Resolution Melting, Berries, Food adulteration.

PL-12

GEOCHEMICAL ATLAS OF THE REPUBLIC OF MACEDONIA

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Beside the research activities on heavy metal pollution in specific areas in the Republic of Macedonia, information about soil quality on a national level were limited. Therefore, a geochemical investigation of soil across the whole country was performed to address this information deficit with this first Geochemical Atlas of the Republic of Macedonia [1]. In this Atlas, the basic geochemical properties of soils are described, as revealed by a detailed large-scale survey across the country and analyses of the findings. It provides the Republic of Macedonia with a sound, well-structured baseline of soil geochemical properties relevant to sustainable land use and soil management and to environmental, agricultural and health-related pressures. The Atlas includes soil sampling and analysis from 1,024 locations with a grid of 5×5 km distance between the sampling locations. Each sample represents a mixture of five subsamples collected in an area of 10 m² to the depth of 0–30 cm. Areas which are known as polluted areas (containing mines, metallurgical factories or larger cities) are investigated taking additional samples on a much denser sampling grid. All samples are analysed for contents of about 50 elements. For this purpose, several sophisticated analytical techniques are applied: inductively coupled plasma – atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS), inductively coupled plasma – mass spectrometry (ICP-MS) and neutron activation analysis (NAA).

After data processing, all important statistical data are presented including: descriptive statistics of measurements with many statistical parameters for 39 elements (Al, As, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Fe, Hf, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, Sb, Sc, Sn, Sr, Ta, Th, Ti, Tl, U, V, W, Y, Zn and Zr), as well as data from multivariate factor analysis and cluster analysis. A distribution maps over the entire territory of the Republic of Macedonia with accompanying text description followed with a tables and histograms with data for the mean, median, minimal and maximal values according to the statistical region in the country, 15 geological formation and 13 pedological units are prepared for each element. The main goal of the Atlas is to define a geochemical background which could be used for further studies and general monitoring the balance between various geochemical factors, particularly those which are connected with anthropogenic soil pollution.

Keywords: Geochemical atlas, soil, pollution, Republic of Macedonia.

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PL-13

INDUCTIVELY COUPLED PLASMA SPECTROMETRY FOR THE ANALYSIS OF ENGINEERED IRON NANOPARTICLES

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Engineered nanoparticles have special physical and chemical properties that differ from larger substances of the same composition and therefore, they have gained the greatest interest in wide areas of materials production and use. Analytical applications are mainly focused to utilize nanomaterials for preconcentration and extraction procedures [1]. Due to large surface area, durability, corrosion resistance, and cost effectiveness, zero-valent iron nanoparticles (nZVI) demonstrated the great potential in removing of specific hazardous substances, such as heavy metals and PCBs from water solutions [2]. Removal efficiency is often determined by imaging of solid particles by SEM or XRD methods, while studies that concern the changes in treated solutions are rarely described.

Analytical performances of inductively plasma optical emission spectrometry (ICP-OES) provide sensitive simultaneous measurements of different emission lines in solutions of complex matrix and therefore, it was adopted in our study of nZVI applications. Synthesis of nZVI particles was performed in ethanolic medium by the method of ferric iron reduction using sodium borohydride, along with subsequent functionalization by different organic ligands. A systematic characterization of nZVI was obtained by XRD and SEM studies. The matrix effects of bare and coated nZVI particles, which denotes to coexisted iron released from nanoparticles into solution, were studied using ICP-OES method. The extraction capabilities of nZVI were tested and optimized to mass fraction used in the experiments on model aqueous solutions containing hexavalent chromium species. The sensitive plasma spectrometry measurements of starting and residual chromium content showed that functionalization of nZVI particles has improved the removal efficiency of Cr (VI) from aqueous solutions.

Keywords: Chromium (VI), ICP spectrometry, Zero-valent iron nanoparticles.

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PL-14

CHEMISTRY, ENVIRONMENTAL FATE AND TRANSFORMATION OF HEXACHLOROCYCLOHEXANES INTO ECOLOGICALLY ACCEPTABLE DERIVATIVES

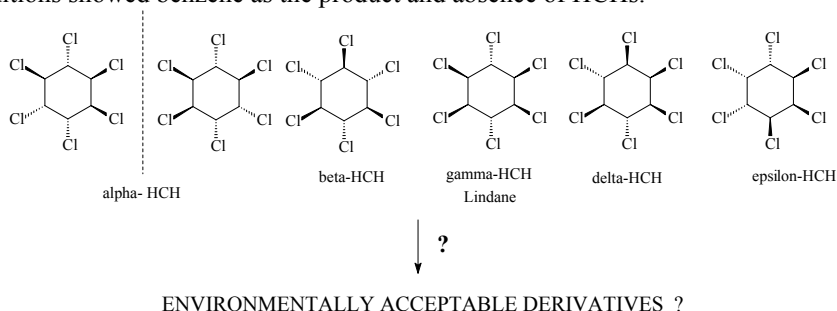
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Hexachlorocyclohexanes (HCHs) and their main representative lindane (γ -HCH) were produced worldwide in the second half of 20th century and were mainly used for agricultural purposes and pest control. Estimates show that between 1.7 and 4.8 million tons of HCH residuals might still be present worldwide. This is also relevant for R. Macedonia, where an estimated 35000 tons of HCHs are located in the factory OHIS. The technical HCH consists of γ -HCH (10–18%), α -HCH (53–70%), β -HCH (3–14%), δ -HCH (6–10%), and ϵ -HCH isomer (1–5%). Also, certain amounts of heptachlorocyclohexane and octachlorocyclohexanes may be present. Depending on the industrial processes and the stage of production, the HCHs waste dump sites can be of different composition, which in turn may require different remediation treatment and sample preparation before appropriate analysis. The different HCH isomers have different physical and chemical properties and have different persistence towards environmental degradation. Lindane and the HCHs are persistent organic pollutants (POPs), and are classified by WHO as “moderately hazardous”. The environmental burden of HCH residuals is still immense and disposal and remediation techniques have been extensively studied.

Herein, brief overview of the chemistry, environmental fate and bulk removal approaches will be given. All of these approaches require proper instrumental methods for monitoring the fate of HCHs and the corresponding products. The focus of our efforts was on dehydrohalogenation and also dehalogenation using metal mediated approaches. GC-MS methods for analysis were developed for assessment of the initial samples from actual waste dump sites and subsequent analysis of the reaction mixtures after treatment. For certain samples of HCH waste, treatment using the optimized conditions showed benzene as the product and absence of HCHs.



Keywords: Hexachlorocyclohexanes, environmental fate, dehalogenation, GC-MS.

PL-15

THE USE OF HPLC METHOD IN ANALYSIS OF MULTICOMPONENT DRUGS

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It is known that single-drug therapy focusing a particular target is no longer considered to be an optimal treatment of multiple complex diseases. Advantages of multicomponent pharmaceutical products are due to multi-targeting, fixed-dose ratio in drug combinations, which at the same time have the ability to reduce the multitude of risk factors without increasing the rate of adverse effects. Basic concepts in the development of some fixed dose drug combinations can be considered the treatment of two related diseases and the inadequate treatment of certain categories of patients with optimal doses of monotherapy [1].

A special chapter in the development of combined pharmaceutical products is their analysis and standardization. Often, pharmacological analytical standards and procedures may not be available for such medicinal products, and new analytical methods need to be developed. Most of fixed-dose active principle drug multicomponent drugs can be analyzed by the HPLC method due to several advantages such as speed, specificity, accuracy and ease of automation in this method. The HPLC method eliminates extraction and isolation procedures, which inevitably lead to loss of active principles and dosing errors. [2] The use of the HPLC method reduces the analytical time, the amount of samples required and the volume of mobile phases used, thus allowing a more efficient analysis. However, there are also a number of challenges in the development of techniques, related to the number of compounds determined and the variety of physico-chemical properties of the compounds: chemical structure, molecular weight, pKa values, UV absorption, concentration of active principle in the analyzed sample, solubility of compounds. Difficulties arise in selecting separation conditions and optimizing them, studying the impact of pH and solvent polarity, selecting the ratio between the aqueous and organic phases [3]. Within the laboratory "Analysis, Standardization and Control of Medicines" of the Scientific Drug Center were recently developed four HPLC techniques for the simultaneous determination of the active principles of mechanical mixtures (at the pre-formulation stage of combined capsules of three active components: piracetam, nicergoline and dry hawthorn extract) and pharmaceutical formulations (ointments containing isohydrofural, methyluracil, benzocaine; auricle drops containing ciprofloxacin, loratadine, dexamethasone and basil volatile oil) which have been validated. These techniques can be included in the quality specifications for these products.

Keywords: HPLC, multicomponent drugs, analysis.

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PL-16

QUALITY ASSESSMENT OF THE DOCTORAL SCHOOLS IN THE FIELD OF BIOANALYSIS

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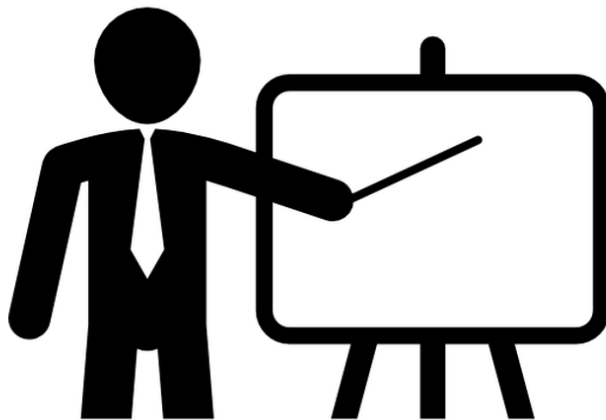
Quality assurance and doctoral education have been elements of the Bologna Process. In 2010, EUA launched the Salzburg II Recommendations that stated that “In order to be accountable for the quality of doctoral programmes, institutions should develop indicators based on institutional priorities such as individual progression, net research time, completion rate, transferable skills, career tracking and dissemination of research results for early stage researchers, taking into consideration the professional development of the researcher as well as the progress of the research project”. This presentation is focused on the “main” indicators that can be used in the bioanalysis field doctoral schools assessment. A schematic pathway of the whole process is discussed.

Keywords: quality assessment, doctoral schools, bioanalysis

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ORAL PRESENTATIONS



O-01

ENHANCED BIOAVAILABILITY OF DRUGS BY NANOFIBERS

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In the last years the natural and artificial polymers were often used in pharmaceutical technology as nanostructural drug carriers, due to their capacity to enhance the bioavailability of poorly soluble active compounds.

The Carbopol polymers are polyacrylic acid derivatives, in which the linear polymer fibers are cross-linked by divinyl-glycol. By copolymerization of these with other polymers (PVP, PVA, CMC) nanofibers with excellent bioadhesivity can be obtained. These nanofibers can be used as drug carriers, and due to the increased specific surface they can enhance the drug release.

The literature gives several methods for nanofiber preparation. The Carbopol nanofibers can be prepared by two methods: the high-speed rotary spinning or centrifugal spinning, and the electrospinning.

The physico-chemical and mechanical properties of drug carrier nanofibers can be studied at the same time using texture analyzers.

The drug release studies showed an increased bioavailability of active compound using these carriers.

Keywords: nanofibers, drug carriers, bioavailability.

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O-02

MICROCHIP ELECTROPHORESIS IN BIOANALYSIS

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Microchip electrophoresis (MCE) has significant benefits in terms of high-speed, high separation efficiency, high-throughput, easy automation, and low sample consumption. The use of MCE in bioanalysis is, however, limited due to a high complexity of biological matrices. For example, short separation channels require injection of relatively simplified samples otherwise separation capacity could be critical together with enhanced risk of peak overlapping. In addition, reduced I.D. of the microchip channels makes heavy demands on detection techniques in terms of sensitivity. Sample clean-up and/or analyte pre-concentration by suitable pre-treatment technique is therefore required. Sample preparation based on electrophoretic principles offers the highest degree of compatibility with MCE and can be on-line combined on the column-coupling (CC) microchip.

In this context, the benefits of the use of CC technology on the microchip combined with various detection techniques (conductivity detection, Vis spectrometry, surface enhanced Raman spectrometry and ion mobility spectrometry) will be presented on a set of analytical methods suitable for rapid monitoring and analysis of complex pharmaceutical and biological samples, e.g., (1) microchip isotachopheresis (ITP) of active ingredients and counter ions in pharmaceutical products [1], (2) zone electrophoresis (ZE) determination of biomarkers with ITP clean-up of body fluids [2], (3) ZE determination of oxidative stress marker in urine, and (4) ITP separation and identification of pharmaceutical additives in antipyretics, etc.

Keywords: Microchip electrophoresis, detection techniques, column-coupling technology, on-line sample clean-up, biological and pharmaceutical samples.

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O-03

MICROCHIP ELECTROPHORESIS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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A new method based on an on-line combination of isotachopheresis with capillary zone electrophoresis (ITP-CZE) has been developed for the analysis of three nonsteroidal anti-inflammatory drugs (NSAIDs) in pharmaceutical products using microchip with coupled channels with implemented conductivity detection. NSAIDs (salicylic acid, diclofenac and ibuprofen) studied in this work are among the most commonly used drugs to treat fever, inflammation, and pain [1]. Used above the therapeutic levels, these drugs can cause a wide variety of adverse effects and their fast analysis could have a significant impact in treatment and recovery of the patients [2]. Optimal ITP separation conditions were achieved with a leading electrolyte at pH 6.5. CZE separations were carried out at pH 7.0 using TES buffer. Detection limits for the studied analytes were in the range from 0.55 to 1.45 mg/L. RSD values of migration times of the analytes present in model samples were within 1.3% and RSDs for peak areas were in the range 0.2–7.6%. Eleven samples of pharmaceutical preparations were analyzed for a content of selected NSAIDs by proposed ITP-CZE method on the microchip. Recoveries of analytes in real samples were in range from 90 to 110%. The developed microanalytical method is suitable for fast (total analysis time was less than 15 min.) and reliable determination of the content of NSAIDs in various pharmaceutical products and can be used as a screening method for quality control purposes.

Keywords: Microchip electrophoresis, conductivity detection, salicylic acid, diclofenac, ibuprofen, pharmaceutical preparation.

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O-04

**SWEEPING OF CHARGED ANALYTES BY NEUTRAL
CYCLODEXTRIN IN CAPILLARY ELECTROPHORESIS**

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Capillary zone electrophoresis suffers from lower detection sensitivity, when compared to high performance liquid chromatography. In capillary electrophoresis, sensitivity of detection can be improved by so-called preconcentration techniques. These techniques utilize physicochemical phenomena to affect electrophoretic velocities of analytes and slow them down thus make analytes zones thinner and more concentrated. Sweeping preconcentration techniques are based on interaction with pseudostationary phase, usually charged micelles or charged cyclodextrins (CDs).

We explored preconcentration technique based on sweeping with neutral CD. The technique was characterized by both computer simulations and experiments and theoretical preconcentration factor was derived. Conductivity detection was utilized with advantage of fully predictable signal enhancement. PeakMaster 5.3. software serve as powerful tool for optimization of electrophoretic systems with respect to maximal enhancement factor and thus lowest detection limits within the sweeping by neutral agent. Rearrangement of system zones and creation of new ones at CD boundary caused by analyte passing thru was observed and their contribution to conductivity signal enhancement was explained.

Keywords: online preconcentration, sweeping, cyclodextrin, conductivity detection.

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O-05

¹H MRS METABOLIC QUANTITATION METHODS IN BRAIN CHEMISTRY

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Hydrogen 1 (¹H) magnetic resonance (MR) spectroscopy allows noninvasive in vivo quantification of brain metabolite concentrations which together with the presence of macromolecules and lipids contain relevant information for diagnosis purposes. At present, metabolite concentrations are more often presented as ratios rather than as absolute concentrations and this may introduce errors and can lead to misinterpretation of spectral data and to incorrect concentration values [1]. Another problem connected with reliable quantification is a metabolite peak overlapping which causes spectral line distortion [2].

In our work, we used raw single and multivoxel spectroscopy data from healthy volunteers and patients with brain tumors, multiple sclerosis and process of demyelination. All spectra were preprocessed with different preprocessing techniques to enhance spectral quality. Various time and frequency domain absolute quantitation methods incorporated in jMRUI software [3] package were used, which impose black box or prior knowledge quantitation methods (HLSVD, AMARES, AQSES).

Another approach, spectra deconvolution and band shape analysis spectra differentiation were carried out to find hidden and location of overlapped peaks.

Keywords: ¹H MRS, brain metabolite, absolute concentrations, AMARES, AQSES, band shape analysis.

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O-06

ASSESSMENT OF CHANGES IN FREEZE-DRIED PROTEIN PHARMACEUTICALS

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Protein pharmaceuticals are becoming an important class of drugs. Due to their complex structure and instability in solution, their development is often challenging [1]. Numerous attempts have been made to increase the long-term stability and shelf life, with freeze-drying as one of the most exploited methods.

The design of freeze-dried protein pharmaceuticals includes various methods of assessment of the changes that occur both during and after the process, and various analytical techniques can be exploited. Differential scanning calorimetry can be useful in determination of T_g, T_g', and crystallization behavior in the frozen state [2]. The degradation is usually monitored using size exclusion chromatography or cation-exchange chromatography, but also electrophoresis can be used in order to detect presence of species with different molecular weight. The changes that occur in the secondary and tertiary structure of various proteins in both pre- and post- freeze drying have been investigated using a number of techniques including FTIR, Raman, NIR, fluorescence, phosphorescence spectroscopic techniques as well as NMR [3-5].

In this work we give an overview of the most frequently applied analytical techniques, as well as our experience in assessment of the changes in freeze-dried rituximab antibody immunoconjugates.

Keywords: freeze-drying, antibodies, analytical techniques.

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O-07

**DEGRADATION PROFILE OF ZEIN NANOPARTICLES AS
TARGETED DELIVERY SYSTEMS**

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Nanoparticles (NP) with well-defined properties can lead to a more rational use of pesticides, allowing a targeted and sustained delivery of the active compound for crops [1]. A promising source of NPs is zein, a protein obtained from corn endosperm. Its hydrophobic character and ability to self-assemble into NPs can be exploited for the incorporation of hydrophobic compounds [2].

The present study aims to investigate the weathering and fate of zein NPs under environmentally relevant conditions. Several cationic and non-ionic surfactants able to induce different surface properties were tested, such as didodecyldimethylammonium bromide (DMAB), polysorbate 80 (Tween 80), nonaethylene glycol monododecyl ether (NEGMDE) and n-dodecyl β -D-maltoside (DBDM), respectively. Hydrodynamic size and surface charge measurements were performed on various freeze-dried zein based NP samples, followed by accelerated degradation studies. Degradation profiles were obtained at extreme soil pH values (pH 4 and 9) for every type of tested NP system. The changes in time of the parent compound were monitored by capillary gel electrophoresis with UV detection, where the rate constants of their hydrolytic degradation allowed the approximation of zein NPs persistence in aqueous media at a chosen temperature.

The data provided by the current study are critical for the assessment of NPs' degradation and offers a better understanding of the surfactants' impact on zein nanodelivery systems.

Keywords: nanoparticles, zein, targeted delivery, degradation, electrophoresis.

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O-08

CHARACTERIZATION OF A POLY(DIMETHYLSILOXANE) MICROFLUIDIC CHIP CONTAINING IMMOBILIZED TRYPSIN FOR RAPID PROTEIN DIGESTION

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Peptide mapping is a method used during the identification of proteins or the analysis of their primary structure or posttranslational modifications. It includes the enzymatic digestion of the sample and the separation/detection of the peptide fragments. The most time-consuming step of peptide mapping is enzymatic digestion, since the enzyme (trypsin) can only be applied in low concentrations (protein:trypsin = 20-100:1) due to its self-digestion. The autolysis of trypsin can be suppressed by the immobilization of the enzyme onto a solid support. In this work, we characterize an immobilized trypsin microreactor integrated into poly(dimethylsiloxane) (PDMS) microfluidic chip.

A PDMS microfluidic device (MD) consisting of several serpentine channels was fabricated by means of soft photolithography. Trypsin layer adsorbed on PDMS was characterized via atomic force microscopy (AFM) and surface plasmon resonance (SPR) spectroscopy. The enzymatic activity and efficiency of the reactor was investigated by digesting protein samples, the obtained peptide mixture was analyzed using capillary electrophoresis (CE) and mass spectrometry (LC-MS/MS). High tryptic activity was maintained within 2 hours after adsorption and then it gradually decreased. To the best of our knowledge, this is the simplest possible immobilization technique which makes the MD an attractive alternative to other immobilized enzymatic reactors [1].

Covalent immobilization via carbodiimide activation was also applied to form a different type of reactor [2]. Immobilized trypsin silica beads were slurry packed into PDMS microfluidic chip, without the use of frits, but with simple bottlenecks. The reactor's reproducibility and efficiency was examined with the digestion of protein samples and the analysis of the digests by CE and LC-MS/MS.

Keywords: trypsin immobilization, IMER, peptide mapping.

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O-09

**CHEMICAL CHARACTERIZATION OF STATIONARY PHASES
FOR FAST LIQUID CHROMATOGRAPHY**

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The retention behaviour of octadecyl-silylated (C₁₈) silica stationary phases with different packing structure, such as monolithic, core-shell or fully porous packings were characterised using methods formerly introduced by Tanaka and his group. Thereby the amount of alkyl chains, hydrophobicity, steric selectivity, amount of silanols and hydrogen bonding capacity of six different chromatographic columns were evaluated.

Since modern silica materials are of high-purity, and accordingly the stationary phases show decreased peak tailing caused by the activity of acidic residual silanols, the employed test compounds need to be selected carefully.

For the comprehensive examination of the silanol effect on the base-deactivated stationary phases, the afore-mentioned methods were used with compounds that are more sensitive for the silanol activity, too. Based on the results, we can conclude that the sensitive test compounds can differentiate between the various types of modern stationary phases

Keywords: C₁₈ silica, chemical characterization; silanol effect; sensitive test compounds.

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O-10

SIMPLE AND RAPID DETERMINATION OF BIOGENIC AMINES IN WINE BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY (UPLC TQ/MS)

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Simple, rapid and sensitive analytical method for direct determination of biogenic amines tryptamine, putrescine, histamine, phenylethylamine, tyramine, cadaverine, spermine and spermidine in wine has been developed and validated. Detection of analytes was performed with ultra-performance liquid chromatography (UPLC) coupled to triple quadrupole mass spectrometer (TQ/MS). To evaluate the matrix effect, the signal suppression enhancement (SSE) was calculated as the ratio of the slope of the standards in wine matrix and the slope of the standards prepared without matrix for each biogenic amine. A negative influence and ionization suppression causing decrease of the signals was observed for spermidine (SSE: 95.1) and histamine (SSE: 90.6), whereas increased signals were observed for cadaverine (SSE: 131), putrescine (SSE: 114), tyramine (SSE: 116), tryptamine (SSE: 109), and no influence was observed for 2-phenylethylamine (SSE: 100). The calibration curves of all amines were linear with correlation coefficients (R^2) > 0.9906. The accuracy of the method was checked with a standard addition method, showing good accuracy, repeatability and reproducibility ($RSD < 10\%$). The limit of detection (LOD) and limit of quantification (LOQ) ranged from 0.50 to 30 $\mu\text{g/L}$ and 1.50 to 90 $\mu\text{g/L}$, respectively, for all amines. The validated method was applied to detect and quantify biogenic amines in Macedonian red and white wines [1].

Keywords: wine; biogenic amines; validation; UPLC-TQ/MS.

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Acknowledgement: Financial support provided by the Macedonian Ministry of Education and Science for the bilateral project between R. Macedonia and Montenegro titled: "Biogenic amines and aroma in Vranec wines from Macedonia and Montenegro and effect of malolactic fermentation on their formation" is gratefully acknowledged.

O-11

**MALDI-TOF CHARACTERISATION OF BOTANICAL
EXTRACTS FOR ENOLOGICAL APPLICATIONS**

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Enological tannins derived from selected botanical sources are routinely used in winemaking as fining coadjutant (International Code of Oenological Practices, 3.2 CLARIFICATION OF WINE), while further applications in foods and engineering have been developed. The chemistry of tannins in wine is mainly affected by their size, solubility in hydro-alcoholic system, their redox potential, and their interaction with proteins and biological molecules [1].

In this study, a selection of commercial oenological tannins extracted from grape, quebracho, green tea, gallnut, oak and chestnut, was profiled for their composition in monomers and polymeric fractions (phenolics, polysaccharides, lignin residues) using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) spectroscopy. Although the general composition showed a variability related to their botanical sources, the extractable fraction was generally composed by monomeric and oligomeric phenolic compounds, together with glycosylated fragments and degradation by-products of organic substrates.

The MALDI-TOF technique is a valuable tool for the qualitative analysis of enological tannins, through the identification of distinctive botanical features on a molecular basis [2,3]. The present work aimed to support the information obtained by mean of analytical techniques routinely used for the compositional study of tannins. Molecular fingerprints for authentication have been highlighted, and a prediction of their sensory and technological impact has been attempted considering the possible structure-activity relationships.

Keywords: Oenological tannins, MALDI-TOF, Molecular patterns, Tannins impact in wine.

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O-12

INFRARED SPECTROSCOPY FOR THE PREDICTION OF PHENOLICS IN WINE

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Fourier transform infrared (FTIR) spectroscopy provides rapid and nondestructive analysis of wine, with almost no sample preparation. FTIR spectra contain many information, as a consequence the same spectra may be used to calibrate for many types of constituents. Application of this technique to the evaluation of several chemical/functional parameters in wine, such as color components, antioxidant capacity, colloidal stability, tannins characteristics and sensory score have been reported [1–6]. In this study, ATR-FTIR measurement was used for the prediction in wine of red color components (total and simple anthocyanins, co-pigmentation, small and large polymeric pigments), total and simple polyphenols and tannins. With this aim spectral data were processed and identification of vibrational modes in the fingerprint regions which provide detailed information about skeletal structures and specific substituents was carried out by means of Partial Least Square algorithms through both calibration and cross-validation step. For each phenolic several models were obtained, either considering the full mid-infrared region spectrum ($4000 - 700 \text{ cm}^{-1}$) or the spectral variables at specific wavelength intervals. Accuracy of the calibrations and cross-validation performances were evaluated by means of statistical indexes: R^2 , r^2 , SEC, SECV, RMSEC, RMSECV, SEP, RMSEP, RPD and RER. The study reinforces the knowledge about adoption of FTIR based technique for a rapid evaluation of parameters of enological interest.

Keywords: FTIR, wine, phenolic components prediction, quality control.

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O-13

**IN VITRO PROFILING OF MICROBIAL METABOLISM OF
PHENOLIC ACIDS USING LC-MS/MS**

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Dietary polyphenols and among them chlorogenic acids are receiving much attention due to their beneficial influence on human body. Their activity is ascribed to their antioxidant, anti-inflammatory, anticancer, cardioprotective and neuroprotective properties. In the recent years there is a big interest in understanding the structural changes occurring during their absorption in human body. The majority of those transformations is strongly correlated with microbiota in colon.

The aim of these studies was to describe the influence of three different species of commercially available probiotic bacteria: *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* 299v, *Lactobacillus reuteri* DSM 17938 on three polyphenols: chlorogenic acid (5-caffeoylquinic acid), caffeic acid and ferulic acid. For that purpose standards of phenolic acid were incubated with solution of bacteria in 37°C in anaerobic conditions. Samples were collected every hour during 7 hours and after 24 hours. LC-ESI/MS/MS was introduced to qualitative and quantitative analysis.

No differences were observed during the incubation of phenolic acids with *Lactobacillus rhamnosus* GG. In contrary, *Lactobacillus plantarum* 299v caused degradation of caffeic and ferulic acids to dihydrocaffeic and dihydroferulic acid. Fermentation–time profiles of analyzed acids showed that ferulic acid was more stable than caffeic acid. For caffeic acid also other degradation products were observed. Ester bound in chlorogenic acid was resistant to the *Lactobacillus plantarum* 299v. *Lactobacillus reuteri* DSM 17938 caused the degradation chlorogenic acid to caffeic acid and quinic acid. Caffeic acid and ferulic were stable during the incubation with that bacterial strain.

Our measurements show that commercially available probiotics are good source of active bacterial. What is more, *Lactobacillus plantarum* 299v and *Lactobacillus reuteri* DSM 17938 are involved in the transformation of phenolic acids. The rate and extent of degradation of phenolic acids showed a clear structural dependence as well as a significant influence of the bacterial strain.

Keywords: phenolic acids, microbiota, metabolism, LC-MS/MS.

O-14

OPTIMIZATION OF SAMPLE PRETREATMENT PHASES IN GC-MS ANALYSIS OF FATTY ACIDS

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Fatty acids are key components of human membranes, being involved directly and indirectly in almost all metabolic processes. Their profile has been proved to be modified in different pathological conditions, such as cardiovascular diseases, neurological diseases or cancer. For this reason, our aim was to develop a fast and efficient separation method for fatty acids in human serum.

The low consumption of volumes, the relative low analysis times obtained in gas chromatography (GC) and its high efficiency coupled with the high selectivity and sensibility of the mass spectrometer (MS) make this the optimal approach for the analysis of fatty acids, while the derivatization of fatty acids to their methyl esters (FAME) is relatively simple and fast.

The present developed method was firstly optimized regarding the analysis time. For these analyses, a commercial standard containing 37 FAMEs was used. The GC-MS separation was done using a non-polar column (HP-5ms, 30m, 0.25mm, 0.25 μ m). An analysis time of 35 min was obtained with quantifiable resolutions for most fatty acids.

The pretreatment of samples basically consists of: extraction of fatty acids (in case of real samples), derivatization, extraction of FAME. The derivatization was always made with methanolic HCl at 70°C, for 3 h. The tests consisted in the following procedures: derivatization followed by SPE, derivatization followed by LLE, derivatization-LLE combined in vial followed directly by injection. The yields of derivatization were calculated after constructing calibration curves using FAME standards which were directly injected into the GC-MS system.

Results have shown significant differences between the three tested procedures. In conclusion, we have developed a faster and fully optimized GC-MS method for the separation of fatty acids, which can also be successfully applied on human real samples.

Keywords: fatty acids, FAME, gas chromatography, yield of derivatization, separation, calibration.

O-15

EXTRACTS OF FOREST HERBS AND FRUITS – THE ANTIOXIDANT ACTIVITY AND THE CONTENT OF BIOPHENOLS

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Heather (*Calluna vulgaris*) and wild berries (*Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L.) are dominant species of forest plants growing in Poland. They are rich source of flavonoids, phenolic acids, anthocyanins, stilbenes and tannins, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins and minerals [1, 2]. Traditionally, *calluna vulgaris* (L.) hull is used in folk medicine for treat urinary tract disturbances and against the common cold and rheumatoid arthritis [3]. Berries fruits are used to treat disorders of the gastrointestinal tract and diabetes [1, 4]. Heather and wild berries samples were collected in three different locations in central Poland (the Mazovia plain). The extracts were prepared in water and ethanol-water mixture.

The extracts from each forest plant were evaluated for the determination of total phenolic content using Folin-Ciocalteu assay and DPPH radical-scavenging activity. The content of flavonoids was determined by spectrophotometric methods based on the formation of Al(III)-flavonoid complexes and the total content of anthocyanins was estimated according to pH differential method. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS) was used to determine the concentration of some biophenols.

Keywords: berries, heather, biophenols, antioxidant activity, HPLC-MS, spectrophotometric methods.

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O-16

**DETERMINATION OF THE POLYPHENOLIC PROFILE OF
SELECTED APPLE CULTIVARS FROM MACEDONIA WITH
LC-MSⁿ**

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Systematic evaluation of the polyphenolic content of 21 commercial, domesticated and autochthonous apple cultivars (*Malus domestica Borkh.*) from Republic of Macedonia harvested during two years (2014 and 2015) was performed in this work using UHPLC-DAD-HESI-MSⁿ. Polyphenolic compounds were separated on a reversed phase UHPLC column with optimized gradient conditions consisting of 1% formic acid in water and 1% formic acid in methanol within 20 minutes, after extraction of the polyphenolic compounds from apple peel, flesh and leaves with methanol/water mixture (90:10, *V/V*). For identification purposes, screening consisting of three MS experiments was implemented; full scan, single ion monitoring and data-dependent scan for the aglycone/glycoside pairs were used as target identification experiments. Additionally, five MS experiments were tested on sixteen reference polyphenolic compounds to optimize the ion trap for quantification so that each class of polyphenolic compounds was quantified employing its own optimized tune against a suitable/available standard. Phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavones, flavonols, flavanols, dihydrochalcones and their derivatives were identified and quantified in all apple samples according to their retention times, UV-Vis maxima and fragmentation patterns. Analytical characteristics of the developed MS method confirmed after validation of the UHPLC-HESI-MSⁿ were comparable to UV-DAD with regards to recovery, precision and robustness, and were superior for its linear range, sensitivity and selectivity.

Keywords: HPLC-DAD-HESI-MSⁿ, apples, polyphenols, peel, pulp, leaves.

O-17

COMPARISON OF VARIOUS REVERSED PHASE STATIONARY PHASES BY MANUAL AND AUTOMATIZED FLOW-REVERSAL METHOD

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In this study, several columns packed with reversed phase (C₁₈) core-shell particles with various particle diameters as well as a silica-based monolithic column were tested and compared.

Lambert et al. used the flow-reversal method with injecting thiourea to characterize the sample band broadening in the chromatographic column and for showing the differences between the two respective column ends. They observed that flow-reversal has a peak compression effect, therefore the peaks observed are always narrower and more symmetrical than the peaks obtained without reversing the flow.

In the present study, peak parking measurements were carried out with human insulin to measure the axial dispersion coefficient and when we define the diffusion coefficient (D_m) of insulin in aqueous solution of acetonitrile with TFA we can calculate the tortuosity of stationary phase.

A small molecule like thiourea can diffuse easily from a fast channel to a slow one, however proteins usually have low diffusivity, therefore we use insulin for our experiments to confirm the multipath dispersion effects. Theoretically if the observed peaks of insulin are also narrower with reversed flow than without reversing flow, one can conclude that the protein molecules travel through the same interstitial channels inward and outward the column.

Keywords: Column-reversal method, Peak parking method, Band broadening, Core-shell particles, Monolith column, Insulin.

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O-18

**CHARACTERIZATION OF LACTIC ACID PRODUCTION
USING *LACTOBACILLUS CASEI* B-26 STRAIN IN DIFFERENT
GROWTH CONDITIONS**

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Lactobacillus casei strains produce lactic acid as their principal metabolic end product. Due to this property they are used in the food industry and as probiotics. The utility of *Lactobacillus casei* is strain dependent, metabolic differences are known among strains as a connotation of the high genetic variation present in this species.

Carbohydrate utilization is one of the factors that play a key role in the industrial utility of *L. casei*. In the present work *Lactobacillus casei* B-26 strain isolated from dairy product was used for lactic acid production under anaerobic fermentation process. Five carbohydrates were used as substrate: D-glucose, D-fructose, sucrose, D-mannose and inulin. The lactic acid production capacity of the *Lactobacillus casei* B-26 strain from different carbon sources was determined. The effect of different carbon source on the fermentation process was done using high performance liquid chromatography (HPLC). The results were used to determine the carbohydrate metabolism in *L. casei* B-26 and to compare with the metabolic network available from literature.

Keywords: *L. casei*, lactic acid, fermentation, carbohydrate.

O-19

**POPPY SEED TEA, A POSSIBLE SOURCE OF MORPHINE
ADDICTION OR OVERDOSE**

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Morphine, an alkaloid found in the poppy (*Papaver somniferum*) is a highly effective pain killer but also one of the most addictive substances known to humans. In order to prevent recreational use of this substance, its use is under strict regulations. Poppy seeds contain morphine in very small amounts and their use as food ingredient is safe; in order to further reduce the morphine content of the poppy seeds they are washed before selling them. However, opioid addicts sometimes wash large amounts of poppy seeds and drink the obtained liquid in order to obtain their dose of morphine. There is a high risk involved by this procedure because extremely large variations could be found in the morphine content of several types of seeds commercially available. This large variation could lead from "nothing happening" to morphine overdose and even death.

We developed and HPLC-UV method with detection at 205nm that could measure very low amounts (1 mg/kg of seeds, concentration far below any toxicological concern) of morphine and codeine in poppy seeds and teas made using different methods published on internet by morphine users.

Concentration of morphine ranged from below 1 mg/kg to as much as 243 mg/kg. The results show that some preparation methods recommended on the internet could easily lead to overdose or death especially in users with low degree of tolerance. No sample contained codeine in concentration high enough to have any risk on poppy seed tea consumers. Such high amounts of morphine found in some type of seeds suggest that regulations regarding the seed washing procedure are not followed by some manufacturers.

Keywords: morphine, codeine, poppy seed tea, addiction, overdose.

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O-20

ISATIS TINCTORIA OR INDIGOFERA TINCTORIA?

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Both plants and insects were used as natural dyes in ancient textiles. Indigo is one of the oldest dyes, known to mankind, used for dyeing textiles in blue. Natural indigo was obtained from two plants: *Indigofera tinctoria* and *Isatis tinctoria*. Although, indigo synthesis begins from different precursors in each plants mentioned before (indican or isatan B), the dye composition, prepared from them is very similar. For this reason, identification of indigo's plant source is often difficult and elusive. In the past, high content of indirubin was considered to be characteristic for *Indigofera tinctoria*. However, according to recent results found in literature the indirubin content in indigo dye prepared either from *Isatis tinctoria* or *Indigofera tinctoria* are indistinguishable. The plants have very similar composition due to distinction between them is problematic and difficult.

The goal of this study is to find a distinctive marker that distinguishes these plants in fabrics. LC-MS/MS was used for the determination of all chemical compounds in dimethyl sulfoxide extracts prepared from fresh, dried or fermented leaves of *Isatis tinctoria*. From each part of leaves the contemporary wool were dyed according to the historical recipes. Regarding to *Indigofera tinctoria*, only dried leaves were available to analyze. The analysis were compared with the results obtained for the commercially available dyes both from *Isatis tinctoria* and *Indigofera tinctoria*. The analysis of plants' extracts allowed to conclude that indigo prepared using *Isatis tinctoria* and *Indigofera tinctoria* contained similar amounts of indirubin. However, the isatin content was significantly higher for *Indigofera tinctoria* than for *Isatis tinctoria*. Also in extracts from contemporary dyed threads the ratio isatin/indigotin is significantly lower when *Isatis tinctoria* was source of blue colour than *Indigofera tinctoria*. The method has been successfully applied to determine the source of the blue dyes in archaeological textiles from the excavations in Sudan.

Keywords: natural dyes, indigo, LC – MS/MS, Sudan.

Acknowledgements: The work was partially supported by "Nubian textiles: craft, trade, costume and identity in the medieval kingdom of Makuria" (NCN POLONEZ 2015/19/P/HS3/02100) and 12000-501/86-DSM-112700.

O-21

STUDY OF CYTOTOXIC EFFECT OF NOVEL AVPI-RGD HYBRID PEPTIDES

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High apoptotic threshold is a major feature of cancer cells and a main reason for these cells' resistance to conventional radio- and chemotherapy. Currently, design and development of a new class of agents affecting critical regulators of apoptosis is a focus of modern target therapy [1]. Synthesis of small peptides and peptide-mimetics comprising functionally different subunits is another promising approach in pharmacology design.

In recent years, many AVPI-, Smac- and RGD- peptide mimetics have been synthesized [2]. AVPI (Ala-Val-Pro-Ile) is a tetrapeptide sequence from the N-terminus of the pro-apoptotic Smac molecule. Binding to several members of IAP protein family (inhibitor of apoptosis proteins), AVPI releases their inhibitory effect and reactivates apoptosis [3]. RGD (Arg-Gly-Asp) is a sequence interacting with proteins over-expressed on the cell membrane of cancer cells.

Herein, we focused on studying the cytotoxic effect of novel AVPI-RGD hybrid peptides. Their cytotoxic potential was determined by MTT assay over 4 cancer cell lines (MDA-MB-231, MCF-7, HepG2, HT-29). Except for one cell line, one of the conjugates showed a higher cytotoxic effect compared to individual subunits used alone, as well as the subunits used in combination.

Keywords: AVPI, cytotoxicity, MTT assay.

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O-22

EFFICACY OF A BIOMONITORING (MOSS) TECHNIQUE FOR DETERMINING HEAVY METALS DEPOSITION TRENDS. CASE STUDY BREGALNICA RIVER BASIN, REPUBLIC OF MACEDONIA

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Application of several moss species (*Hypnum cupressiforme*, *Homalothecium lutescens* and *Scleropodium purum*) for monitoring of anthropogenic impact on heavy metals air pollution in Bregalnica River Basin, Republic of Macedonia, was studied. Moss samples were review for their potential to reflect heavy metals air pollution. Potential “hot spots” were selected in areas of copper mine (Bučim mine) and lead and zinc mines (Zletovo mine and Sasa mine) as main metal pollution sources in the Eastern part of the Republic of Macedonia. Continuously, dust distribution from ore and flotation tailings occurs. This results with air-introduction and deposition of higher contents of certain metals. Determination of chemical elements was conducted by using both instrumental techniques: atomic emission spectrometry with inductively coupled plasma (ICP-AES) and mass spectrometry with inductively coupled plasma (ICP-MS). Combination of multivariate techniques (PCA, FA and CA) was applied for data processing and identification of elements association with lithogenic or anthropogenic origin. Spatial distribution maps were constructed for determination and localizing of narrower areas with higher contents of certain anthropogenic elements. In this way influences of selected human activities in local (small scale) air pollution can be determined. Summarized data reveal real quantification of the elements distribution not only in order determination of hazardously elements distribution, but also present complete characterization for elements deposition in mines environs.

Keywords: moss; heavy metals; biomonitoring; ICP-AES; ICP-MS; multivariate analysis.

O-23

THE EFFECT OF PGP BACTERIAL INOCULATION ON THE ACCUMULATION OF HEAVY METALS IN CROP PLANTS

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Heavy metals can occur in agricultural soils deriving either from natural sources (rocks, sea water, dust, volcanic gases, forest fires) or anthropogenic activities (mineral fertilizers, mining). The presence of these elements in high concentrations can affect plant growth and show toxicity in most plants. Biopreparates based on plant growth promoting (PGP) bacteria are used in sustainable agriculture practice to enhance plant growth of crop plants. Beside the plant growth promoting effect they can also influence the metal uptake of the plants.

The aim of our research was to determine the effect inoculation with PGP bacteria (*Mitsuaria chinosanitabida*) on metal uptake (Cd^{2+} and Zn^{2+}) of crop plants. For this study two crop plants, maize (*Zea mays*) and wheat (*Triticum aestivum*) were used.

The crop plants were grown for 14 days under controlled conditions in a plant growth chamber then harvested and measured (fresh and dry weight and length of root and shoot). The amount of accumulated heavy metal (Zn^{2+} , Cd^{2+}) in these two crop plants was determined using atomic absorption spectrophotometry. We observed no statistically significant difference in plant growth among non treated, bacterial inoculated, heavy metal treated and both bacteria and heavy metal treated plants. Our results showed that the presence of PGP bacteria lowered the accumulation of both heavy metals in both plants. A limited translocation was observed between the root and shoot of both plants for both heavy metals. There were no difference between the essential micronutrient (Zn^{2+}) and toxic heavy metal (Cd^{2+}) translocation in case o the used crop plants.

Keywords: *Mitsuaria chinosanitabida*, maize, wheat, heavy metal, accumulation.

O-24

THE INFLUENCE OF UV-VIS IRRADIATION AND OXIDATION ON ARSENIC AND CHROMIUM SPECIATION IN WATER

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Distinction between chemical forms of arsenic and chromium is a key element in the monitoring of the aquatic environment. It is well known that the toxicity of elements depends on their chemical or physical forms. Depending on the oxidation degree, the effect on organisms is different. Cr(III) is a nutrient, while Cr(VI) is mutagenic and carcinogenic. Chromium mainly occurs in the form of inorganic oxyanions. Cr(III) is less soluble and more stable, while Cr(VI) is more soluble and mobile in the environment [1]. The most toxic forms of arsenic are inorganic compounds: arsenic hydride, arsenates (III) and arsenates (V). Less toxic are organic derivatives of As. Organic arsenic compounds, such as arsenocholine (ASC) and arsenobetain (ASB) are non-toxic [2,3]. Environmental conditions, i.e. intensity of radiation, oxygen content and activity of microorganisms, can change element's speciation and toxicity. Due to large differences in toxicity of various forms of As and Cr, it is important to monitor their total content and speciation in the environment. Arsenic speciation was determined by high performance liquid chromatography (HPLC) with UV-Vis detection. A mixture of standard solutions of arsenic (III) and arsenic (V) was irradiated with solar-imitating light (1.5 AM). Two parallel irradiation procedures were performed in the presence of an active layer. During the irradiation the samples were argoned or oxygenated. Samples were collected after 2 and 6 h of irradiation. Chromium (III) standard stability was also tested for 30, 60 and 90 min of irradiation using a lamp emitting solar-imitating light, in the presence of oxygen or argon. Diethylenetriaminepentaacetic acid (DTPA) was added to the sample to stabilize Cr(III). After irradiation HPLC UV-Vis analysis was performed.

Keywords: photocatalysis, arsenic, chromium, speciation analysis.

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O-25

SORPTION OF Sc(III) ON SOLID CARBON NANOMATERIALS

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Since the use of scandium in industrial applications has been increased growing, the possibility of its release into the environment has also increased. Determination of Sc(III) in environmental samples requires often preconcentration and separation step due to a low metal content and high concentration of the interfering matrix components [1]. Among techniques applied for this purpose, batch and column approaches in which Sc(III) is sorbed on different water-insoluble solid materials and further eluted with acids or complexing reagents have been widely used. Scandium can also be used as a long-lived radionuclide in positron emission tomography [2]. Prior to labeling, separation from target material should be carried out. Solid phase extraction is popular technique for this purpose. Carbon nanomaterials are potential sorbents for both environmental and radiopharmaceutical applications. In this study sorption of scandium(III) on graphene oxide (GO) and oxidized carbon nanotubes (CNT-COOH) in different pH conditions was checked. Static experiments, in which certain amount of sorbent was shaken with scandium(III) ions solution for 24 h were performed. Generally sorption of scandium increased with increasing pH. In the pH range of 2.0–5.5 sorption of Sc(III) onto CNT-COOH is quantitative. For GO pH range for maximum sorption is 2.5–5.5. Both sorbents show fast sorption kinetics. For application in solid phase extraction dynamic experiments in columns were also performed. CNT-COOH sorbent showed optimal characteristics in pH as low as 2.0. Effective separation of scandium from calcium matrix was performed.

Keywords: solid phase extraction, carbon nanotubes, CNT-COOH, scandium.

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O-26

APPLICATION OF 2-D ELECTROPHORESIS AND MALDI TOF MS ANALYSIS FOR THE QUALITY STUDY OF BIOLOGICS

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Biological medicinal products or biologics are complex molecules obtained by the application of rDNA technology. Due to their production process, co- and posttranslational modifications, these drugs are heterogeneous mixtures composed of numerous isoforms. Therefore, in order to evaluate their quality complex analytical methodology, consisting on the combination of more than one technique is required. The aim of this work was to study the suitability of gel electrophoresis and MALDI-TOF-MS analysis for the quality assessment of selected biopharmaceuticals, including therapeutic *rmAbs*, trastuzumab and rituximab, and fusion protein, abatacept. One-dimensional, SDS-PAGE, and two-dimensional gel electrophoresis were used for the determination of molecular mass (Mr), the isoelectric point (pI), charge-related isoform patterns and the stability of selected biological medicines. For the assessment of the influence of glycosylation in the charge heterogeneity pattern, enzymatic deglycosylation study has been performed using N-glycosidase F, sialidase, and O-glycosidase. Peptide spots separated in 2-DE gels were *in gel* tryptically digested, resulting peptides were subjected to MALDI-TOF-MS analysis [1,2]. Experimental data revealed that 1D and 2D gel electrophoresis represent easy methods to evaluate the quality of biological medicinal products. Given that 2-D electrophoresis, MALDI-TOF MS have already matured, equipment is commercially available and no special personnel requirements are needed, this analytical strategy might be used as routine technique in quality control laboratories.

Keywords: rituximab, trastuzumab, abatacept, 2-D electrophoresis, MALDI-TOF.

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O-27

**STUDY OF THE OXIDATIVE METABOLIZATION PATTERN
OF BETA-BLOCKERS**

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In the last decade the mimicking of xenobiotics' oxidative metabolism using electrochemistry coupled to mass spectrometry has been intensively applied. Predictions in the early stages of drug design and various correlations with the reported side effects of molecules undergoing preclinical trials may be conveniently assessed through the monitoring of the emerging stable and/or transient electrochemically oxidized products. These cost-effective and efficient methods that involve the screening of electrochemically generated species for the elucidation of yet unknown reaction mechanisms became a viable tool in the prediction of phase I reactions (mediated by the cytochrome P450 enzymatic system).

This study aims to understand and fully elucidate electrochemical reaction mechanisms of known drugs (propranolol, atenolol, alprenolol, oxprenolol). The separation and detection of the emerging oxidative products was accomplished by coupling capillary electrophoresis with mass spectrometry (ESI-Ion Trap Mass Spectrometry) or direct infusion of the electrolytic products in a high resolution Time-of-Flight Mass Spectrometry. The separation of emerging compounds was performed using optimized conditions that offer full compatibility with the employed mass analyzers. Furthermore, the high resolution tandem mass spectrometry offered additional structural information required for the identification of unknown metabolites.

Similarities and dissimilarities between the electrooxidative and the biotransformation (human liver microzomes) processes following various oxidation patterns (aromatic-hydroxylation, O-dealkoxylation and N-dealkylation) were discussed and interpreted.

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Keywords: beta-blockers, metabolic pathway, oxidation mechanism.

O-28

**THE OPTIMIZATION OF THE BIOAVAILABILITY OF THE
PROPYLTHIOCINOTHIADIAZOLE**

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Propylthiocinothiadiazole is a new biologically active compound synthesized in the Laboratory of Organic and Biopharmaceutical Synthesis of the Institute of Chemistry of the ASM, which has strong antimicrobial activity. It has been shown to be an active inhibitor of the M. tuberculosis enola-acyl reductase (INHA) carrier protein. INHA is one of the key enzymes involved in fatty acid biosynthesis of mycobacteria and is therefore an effective antimicrobial targeting agent [1].

Previous research has shown low toxicity of the investigated compound. Both in the acute and subacute toxicity tests it produced 0% lethality, so it belongs to the low toxicity class, according to the Hodge-Stern (1956) classification of chemicals based on DL₅₀. One of the possible impediments in the development of a drug formulation would be the low bioavailability of propylthiocinothiadiazole, as demonstrated in vivo studies on rats. Thus, research has been done to optimize bioavailability by complexing with cyclodextrins and testing the obtained binary system, and optimizing the solubility by structural modification, obtaining a sulfuric acid salt [2].

Cyclodextrins are cyclic oligosaccharides formed from α -D-glucopyranose units, which are known to be able to form complexes of inclusion with lipophilic drugs or lipophilic drug residues, thereby producing a change in the physicochemical and biopharmaceutical properties of the drugs [3].

It has been established that cyclodextrin-propylthiocinothiadiazole systems have low toxicity. The salt of propylthiocinothiadiazole with sulfuric acid has a very high degree of solubility and an increased absorption at the gastrointestinal level, which makes it possible to explore the possibilities of developing an injectable pharmaceutical form. Both compounds were characterized physically and chemically by IR spectroscopy, NMR, microscopic analysis, DSC, HPLC. Qualitative and quantitative HPLC analysis demonstrated the purity of the substance and its good retrieval in the sulfuric acid salt.

Keywords: propylthiocinothiadiazole, bioavailability, pharmaceutical formulations.

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O-29

NOVEL APPROACH FOR DNA METHYLATION ANALYSIS IN REGULATORY REGIONS OF *MGMT* IN GLIOBLASTOMA

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DNA methylation of cytosine-guanine dinucleotides (CpG) in promoter regions is mainly connected with gene silencing. There is increasing evidence that not only promoter methylation, but also methylation of further regulatory regions e.g. of enhancers contribute to oncogene activation and tumor suppressor silencing in cancer.

In glioblastoma, the activity state of O6-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in decision between treatment with temozolomide or radiotherapy. However, no optimal predictive detection assay exists so far. Our aim is to find suitable regulatory DNA regions for analyzing MGMT activity and investigate their clinical applicability to select appropriate glioblastoma therapy.

Therefore, we bisulfite converted the extracted DNA and amplified specific regions by real-time polymerase chain reaction (PCR). So far, we have used either cheap, fast methylation sensitive high resolution melting (MS-HRM) analysis featuring low limits of detection, or more expensive pyrosequencing (PSQ). While MS-HRM provides the mean methylation status per amplicon by calibration via standards of known methylation status, PSQ is an absolute method, giving information about the methylation level of every single CpG located in the analyzed region.

Our approach is to combine both methods by subjecting the same PCR products first to MS-HRM and, depending on the MS-HRM outcome, to PSQ. The combined method could be used for cheaper clinical pretesting and for early detection of treatment induced changes.

This lecture will explain the role of DNA methylation in cancer and give an overview of our developed methods for cost effective determination of MGMT activity in glioblastoma.

Keywords: DNA methylation, MGMT, glioblastoma, clinical application.

O-30

**DETERMINATION OF MIDAZOLAM AND ITS MAIN
METABOLITES IN URINE AND SERUM SAMPLES BY LC-UV
AND LC-MS**

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Midazolam is a sedative compound routinely used in intensive care patients. Due to its pharmacological properties, i.e. a half-life of about 3 hours and only minimal influence on hemodynamics, its use in sedation is widely accepted, even for long periods. In patients with renal and liver failure, metabolites of midazolam may, however, accumulate, leading to unwarranted prolongation of sedation.

Methods published so far allow the determination of midazolam and its hydroxy metabolites 1-hydroxymidazolam (1-OHM) and 4-hydroxymidazolam (4-OHM), but not of 1-hydroxymidazolam- β -D-glucuronide (1-OHMG) and 4-hydroxymidazolam- β -D-glucuronide (4-OHMG) in one and the same run. 1-OHMG and 4-OHMG are usually determined after enzymatic cleavage to 1-OHM and 4-OHM. We developed two independent methods, a LC-UV and a LC-MS method, allowing the direct determination of midazolam and its four metabolites. Both methods include sample clean-up by solid phase extraction. The lecture will present the two methods and data obtained by their application to urine and serum samples from cardiac surgery patients.

Keywords: Midazolam, Metabolites, HPLC-UV, HPLC.

O-31

**GUEST – HOST INTERACTION STUDIES BETWEEN
PROPRANOLOL AND β -CYCLODEXTRIN AT SOLID/LIQUID
INTERFACE**

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Gold nanoparticles are an excellent platform for selective and/or sensitive bio-detection offering unique optoelectronic and catalytic properties. As such, they continue to attract considerable interest in electrocatalysis and in the design of chemo- and biosensors.

Cysteine is an appropriate agent to refine gold nanoparticles (AuNPs) because it can form complexes with gold ions, increasing the number of nucleation sites and improving the kinetics parameters without major changes in the purity of deposits [1]. Therefore, it can be used to deposit AuNPs on the electrode surface in a more controllable and reproducible manner.

Cyclodextrins continue to be the most used class of chiral selectors in liquid separation techniques. The ability of a large variety of organic and inorganic molecules to enter in the cavity of cyclodextrins allows the formation of stable or transient guest–host inclusion complexes [2]. SERS studies performed on propranolol revealed distinct conformations of its enantiomers physisorbed on the surface of gold nanoparticles as well as considerably different spectroscopic behavior in the presence of β -cyclodextrin [3].

The present study aimed to investigate the interaction at the solid/liquid interface between AuNPs, the enantiomers of propranolol and cyclodextrin using electrochemical techniques. AuNPs were potentiostatically (-0.4V vs. Ag/AgCl, 3M KCl) electrodeposited on the surface of a glassy carbon electrode, in the presence of cysteine. Following the spontaneous adsorption of propranolol's enantiomers on the gold surface β -cyclodextrin was added. The electrooxidation of enantiomers was performed in phosphate buffer (pH=7.00) by differential pulse voltammetry. The considerable differences in the peak potential and current intensity of the two enantiomers would enable a very simple and convenient way in the chiral probing of drugs with potential applications in the biomedical field.

Keywords: gold nanoparticles, cysteine, cyclodextrin, propranolol.

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Acknowledgement: The current research was financially supported by Ph. D Research Project number 1300/61/13.01.2017 offered by Iuliu Hațieganu University of Medicine and Pharmacy, Cluj – Napoca, Romania. Authors are grateful to CEEPUS Network CIII-RO-0010-11-1617 - Teaching and Learning Bioanalysis.

O-32

FOOD ADULTERATION – A TETRAPLEX REAL-TIME PCR ASSAY FOR THE SIMULTANEOUS DETERMINATION OF FOUR FREQUENTLY CONSUMED GAME ANIMALS

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Consumers are increasingly aware of safety and quality issues associated with factory farming, like high levels of antibiotics, and prefer organic and sustainable food. Meat from game animals has always been appreciated for its taste and tenderness. Nowadays, it is even more popular because of its health promoting properties. It has a favorable ratio of omega-6 to omega-3 fatty acids and does not contain drug residues. According to the Codex Alimentarius Austriacus, 38% of the meat content in a “game” sausage has to originate from game animals. However, food producers and retailers might be tempted to increase their profits by substituting more expensive game meat by cheaper meat from domestic animals. Therefore, food control authorities not only need to identify but also to quantify animal species in food.

Previously, a duplex real-time PCR method for the simultaneous determination of the content of roe deer and the sum of red deer, fallow deer and sika deer has been published ^[1]. However, the increasing trend to market deer under their species specific names rather than the comprehensive term “deer” makes species specific methods necessary. Recently, species specific methods for roe deer ^[2], red deer, fallow deer and sika deer have been developed. In order to enhance sample throughput and save time and costs, these four species specific singleplex methods have been combined to a tetraplex assay.

Our quantitative results obtained by analyzing DNA mixtures and model game sausages with known contents of the target animals indicate that the tetraplex assay is suitable to be applied in routine food analysis.

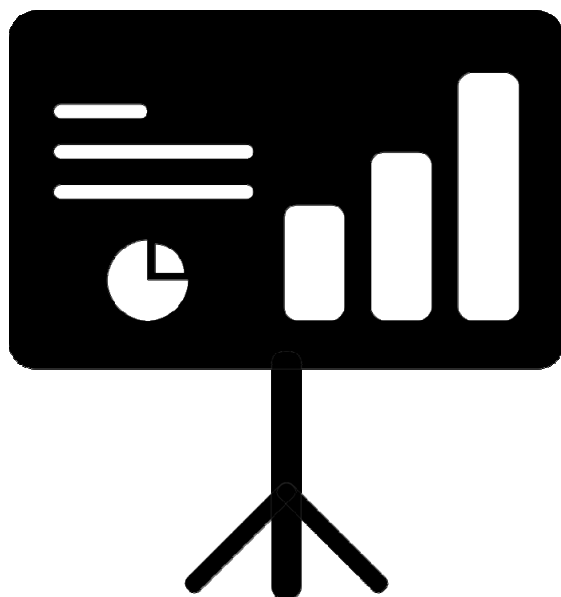
Keywords: Game meat, food adulteration, quantification, multiplex real-time PCR.

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POSTER PRESENTATIONS



P-01

**THE USE OF ELECTROLYTE SYSTEMS COMPATIBLE TO
MASS SPECTROMETRIC DETERMINATION FOR CAPILLARY
ELECTROPHORESIS**

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Capillary electrophoresis (CE) is a widely used analytical technique for the separation of analytes. The advantages of CE are fast separation, small amounts of sample, high resolution and automatization, but identifying the analyte peaks in an electropherogram is quite challenging. However, by using MS detector the different analytes can be determined. Although the coupling CE to MS has some difficulties, CE-MS technology can provide so excellent features that it has been intensively researched during the last two decades [1]. CE-MS method requires an applicable interface thorough electrospray ionization (ESI), volatile buffer system, suitable liquid flow rate and electric connection between the ends of capillary when outlet is connected to the sprayer. Due to the ESI process, the electrolyte system needs to be volatile and the concentration of buffers have to be low to avoid the formation of high currents. Consequently, phosphates and borate generally used in CE can not be applied in CE-MS.

The aim of our study was to find suitable electrolyte systems, which is efficiently applicable either for CE separations and introducing the separated components to MS detector through ESI. For this study electrolytes applied in literature for CE-MS were selected (eg. formic acid, acetic acid, ammonium-acetate and triethylamine). In order to test and compare these “MS-compatible electrolytes” with the phosphate or borate buffers commonly used for CE, peptide mixture obtained by trypsin digestion of human albumin was used for the separations (peptide mapping).

Keywords: peptide mapping, capillary electrophoresis, mass spectrometry, electrolyte systems.

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P-02

**TRYPTIC DIGESTION OF TEAR SAMPLES USING A
MICROFLUIDIC ENZYME REACTOR**

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In our research we developed a microfluidic chip suitable for the rapid and efficient proteolysis of tear samples. The design of the microfluidic chip makes 8 simultaneous digestions possible. The microchip is made of poly(dimethylsiloxane) (PDMS), a supreme porous adsorbent enabling the strong adsorption of large proteins on its surface. Trypsin was immobilized on the serpentine-like channel walls of the microchip through spontaneous adsorption [1]. By applying the surface-bound trypsin in high concentration, we achieved a decrease in the reaction time by orders of magnitude, maintaining the proteolytic efficiency characteristic of the standard, in-solution digestion. In-solution digestion might even take 16 hours, whereas digestion carried out with the microfluidic device takes only 0.014 hours (50 seconds). The digested tear samples were analyzed using capillary zone electrophoresis (CZE). The obtained electropherogram is the peptide map of the given sample, which describes the protein profile of the sample as a fingerprint. Based on the results of comparing the obtained peptide maps, it can be concluded that the designed microfluidic enzyme reactor is capable of reliable digestion, furthermore, the protein composition of tear samples acquired from different individuals may differ.

Keywords: poly(dimethylsiloxane), proteomics, peptide mapping, immobilization, adsorption, capillary zone electrophoresis.

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P-03

ANALYTICAL INSTRUMENTS FOR BIOPHARMACEUTICAL CHARACTERIZATION

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Drugs with biological origin or biopharmaceuticals are products where the active substance is composed of or derived from living organisms and often represent the crossing of the boundaries of medical science and different fields of research (Walsh, 2003). They can be composed of carbohydrates, nucleic acids, proteins, or complex combinations of these substances, or may be composed from live cells or tissues. Unlike chemically synthesized small molecules that have a well-defined structure and are thoroughly characterized, the pharmaceutical products with biological origin are complex in structure and therefore their full characterization is great achievement.

Analytical methodology that is currently in use enables a comprehensive characterization and identification of biopharmaceuticals (determination of molecular weight and particle size, physico-chemical properties, identity, purity and homogeneity, biological activity, immunochemical properties, purity/impurities (if present), present isoforms and structures of higher order, and stability of the product) and covers implementation of variety of physico-chemical, immunological and biological methods (ICH, Specifications Q6B; Tuma, 2005; Wakankar et al., 2011; Gjorgieva Ackova et al., 2015).

Here, we used a multiple set of analytical techniques (HPLC, SDS-PAGE, ICP-MS, MALDI-TOF, TLC, UV/VIS, FTIR and Raman Spectroscopy) for extensive characterization of immunocomplexes of antibody rituximab intended for use as biopharmaceutical.

Keywords: biopharmaceuticals, analytical methods, immunocomplexes.

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P-04

**EVALUATION OF THE REDOX INTERACTION OF RUTIN
WITH SOME MEDICINAL SUBSTANCES BY CYCLIC
VOLTAMMETRY**

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Rutin is one of the most frequently represented flavonoid glycoside found in many plants commonly used in nutrition or herbal preparations. Due to its polyphenolic structure, it could be easily subjected to redox reactions, which allows its analysis by different electrochemical methods.

The aim of this study was to evaluate the interactions between rutin and certain medicinal substances, as a potential food-drug interaction, after consuming them in the same time. These interactions can also lead to some synergistic or antagonistic physiological effects. The interactions between rutin and lorazepam, diazepam, alfa-lipoic acid, glimepiride, acetylsalicylic acid and ascorbic acid, have been evaluated by application of an electrochemical analysis by the means of cyclic voltammetry. The experiments have been performed on a glassy carbon electrode as working electrode and potential range from -0.4 to 0.6 V. The obtained results were used further for calculation of the kinetic of these reactions by implementing the characteristic parameters from cyclic voltammograms in a specially designed theoretical models. The estimated values of the chemical rate constants for the interactions between rutin and the investigated compounds read (in mol⁻¹ L s⁻¹): 0.0025, 0.0022; 0.0024; 0.0020; 0.0550 and 0.0620 for lorazepam, diazepam, lipoic acid, glimepiride, acetylsalicylic acid and ascorbic acid, respectively.

From the obtained results, we can conclude that the highest interaction have been noted between rutin and ascorbic acid. These data can serve into prospecting their *in vivo* interactions.

Keywords: cyclic voltammetry, electrochemical analysis, flavonoid, interactions, rutin.

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P-05

**CYTOTOXICITY ASSESSMENT OF AROYLHYDRAZONE
DERIVATIVES**

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Aroylhydrazones intensively investigated in recent years due to their versatile properties and applications. Many compounds of this type are biologically active as anticancer, antibacterial and antimicrobial agents. In addition, hydrazones as chelating agents investigated as potential drugs for treatment of iron-overload associated diseases.

In the present work, a group of 17 derivatives was studied for cytotoxic activity, as a part of our investigations on aroylhydrazones.

The cytotoxic effect of the compounds was tested on non-tumor cells 3T3 and MCF10 and tumor cell lines HepG2, MCF7 and MDA-MB-231. Compound 11 exhibited the highest growth inhibitory effects on cell lines and significantly higher in tumor cell lines than in non-tumor cell lines. Compounds 5, C and E also show significantly higher cytotoxic activity in tumor cell lines than non-tumor cell lines in the experiment.

The study makes it possible to assess the interaction between the structure and activity of the compounds and to understand the further development of hydrazones as more potent and selective antineoplastic agents.

Keywords: aroylhydrazones, cytotoxicity, MTT assay.

P-06

**SIGNIFICANCE OF THE NON-COVALENT
INTERMOLECULAR INTERACTIONS IN MOLECULAR
COMPLEXES OF PHARMACEUTICAL RELEVANCE**

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The benchmarks that Regulatory Affairs for pharmaceutical product approval imposes over its marketing authorization mainly depend on the biopharmaceutical and pharmacokinetics profile of the Active Pharmaceutical Ingredient (API), that together within excipients, are incorporated into the pharmaceutical formulations. Both of these features are determined by the physicochemical properties that are in correlations with API's structure, additionally affecting its processability, handling and storage. Thus, any combination of API-excipient in blends for their confectionary into pharmaceutical products are exposed to possible non-covalent interactions (mainly H-bonding, $\pi - \pi$ stacking, electrostatic). The nature of these interactions alters the performance of the API (e.g. drug solubility, chemical stability, phase transition, photostability, compressibility, etc.). Therefore, the aim of the concept for "functionalized" excipients, which has been developing for more than the last twenty years, is to utilize favorable intermolecular interactions that affect the formation of distinct solid phase within which molecules of a drug molecule and excipient are non-covalently bounded and that exerts improved properties. Therefore, according to the Guidelines, issued by the ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), and that has been adopted to national regulatory bodies, the focus of drug development process should be in preformulation stage where should be deploying the protocols for screening the new solid phases, their tracking the stability and sustainability of that solid phases during the entire product life-cycle.

The non-covalent interaction is the main driving force for forming inclusion complexes, as well as for forming co-crystals. The advantage of cocrystals, comparing to the properties of inclusion complexes are underlined in terms of participation of H-bonding and its nature to the formation of crystalline phases of carbamazepin cocrystals comparing to amorphous inclusion complexes that cyclodextrins form with the same drug model.

P-07

**USE OF MICROBORE SIZE-EXCLUSION
CHROMATOGRAPHY FOR THE CHARACTERIZATION OF
MACROMOLECULAR SAMPLES**

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Use of small-bore (narrow-bore, microbore) columns in size-exclusion chromatography (SEC) has great significance in achieving the chromatographic separation of polymers and macromolecules with the same molar mass distributions (MMDs) and calibration curves as for conventional SEC columns (8-10 mm i.d.). Generally, the reduction of column internal diameter and gel particle size leads to the higher separation efficiency. Using microbore SEC columns with particle size 10-20 μm then results in substantial decrease of analysis time while maintaining or even increasing the separation efficiency [1]. It has already been demonstrated that small-diameter columns can provide their full efficiency only if the instrument is specially designed for them, meaning particularly reduction of system dispersion, therefore the high equivalent to a theoretical plate is much smaller when using microbore columns with microparticle gels in SEC, resulting in narrower peaks and better resolution.

Keywords: microbore, size-exclusion chromatography, polymers, macromolecules.

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P-08

PRELIMINARY RESULTS OF COUPLING MICROCHIP ELECTROPHORESIS WITH ION MOBILITY SPECTROMETRY

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Ion mobility spectrometry (IMS) is an analytical technique used for the separation and characterization of gaseous ions with respect to their different mobilities in a weak electric field. Main advantages of this technique include fast and reliable analysis, low cost, portability and relatively easy manipulation with IMS instrumentation.

Even though IMS is designated, especially, for analysis of gaseous samples, several different methods have been employed for introduction of liquid samples, e.g., direct injection – thermal evaporation via a heated injector port, nebulizer or by continuous sample flow through vaporizing chamber. In this work direct liquid sampling (DLS) unit was used to overcome problems related with fast evaporation prior to IMS analysis and potential clogging of the heated interface [1]. Corona discharge ionization with point to plate geometry and negative mode of operation was used. Purified atmospheric air was used as a drift gas.

IMS was coupled to microchip electrophoresis (MCE) through DLS unit. Analytes separated on the microchip were hydrodynamically transferred to DLS unit. Developed method was applied to the analysis of organic acids in model and waste water samples. Formic, acetic, propionic, butyric, valeric and caproic acids were analyzed by IMS after their separation on the microchip and followed by a transfer separated analytes to the IMS. Analysis of the acids in waste water samples shows great potential of the developed MCE-DLS-IMS method.

Keywords: microchip electrophoresis; ion mobility spectrometry; coupling; organic acids.

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P-09

**HYBRID PH-SENSITIVE NANOPARTICLES AS PLATFORMS
FOR DELIVERY OF CURCUMIN**

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Curcumin, the yellow powder derived from the plant *Curcuma Longa*, exhibited numerous therapeutic applications against wide range of chronic diseases such as diabetes, pancreatitis, arthritis, neurodegenerative diseases and various types of cancer. The mechanism of antineoplastic activity of curcumin is through modulation of cell signaling pathways, mainly blockage of nuclear factor kappa B (NF- κ B) activation and induction of apoptosis in different types of human cancer cell lines, associated with excellent safety profile. Unfortunately, the tremendous therapeutic potential of curcumin as a chemopreventive, antineoplastic and chemosensitizing agent has failed to progress towards clinical development and commercialization due to its unfavorable physicochemical properties, low aqueous solubility, chemical instability, and pharmacokinetics. An intriguing strategy to overcome these limitations is the design of nanosized vehicles for efficient delivery of curcumin. The present contribution is focused on elaboration of hybrid pH-sensitive liposomes based on dipalmitoylphosphatidylcholine:cholesterol (DPPC:CHOL) and a pH-sensitive poly(isoprene-*b*-acrylic acid) (pI-pAA), whereby curcumin is entrapped as a free drug and as a water soluble inclusion complex with polyoxyethylated calix[4]arene, which allows the drug to occupy both the phospholipid membranes and the aqueous core of liposomes. Nanoparticles were characterized by DLS, cryo-TEM, curcumin encapsulation efficacy and in vitro release as a function of pH. Size, size distribution and zeta potential were evaluated by DLS and the results revealed particles of app.180 nm with monomodal distribution (PDI below 0.2) and zeta potential of – 20 mV suitable for systemic application. The hybrid pH-sensitive liposomes showed high loading efficacy (98%) and improved drug release profile. The excellent in vitro biocompatibility profile and the favorable physicochemical and drug loading characteristics of the tested nanoparticles, and their ability to retain the intrinsic pharmacological properties of encapsulated drug they could be considered promising drug delivery platforms for lipophilic curcumin.

Keywords: curcumin, nanoparticles, complex, encapsulation.

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P-10

**FREEZE DRIED KIT FORMULATION OF TRASTUZUMAB
IMMUNOCONJUGATES**

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Trastuzumab is a humanized anti-HER2 monoclonal antibody used for therapy of metastatic breast cancer. Conjugated antibodies with toxins, drugs and radionuclides, provide high hope for development of cancer-specific cytotoxic reagents. The formulation of stable immunoconjugates with a bifunctional chelators (BFCA) is required in order to obtain successful radiolabeling. The aim of this examination was to development a stable freeze dried trastuzumab immunoconjugates with BFCA p-SCN-Bn-DTPA, p-SCN-Bn-DOTA, 1B4M-DTPA.

Trastuzimab was purified from Herceptin® (Roche) with six cycles of ultrafiltration, by washing the antibody with 0.1M PBS, pH=8. The conjugations was made by mixing the antibody with 10 mg/ml solution of BFCA in different ratio (p-SCN-Bn-DTPA – 1:10; 1:20; 1:50, 1B4M-DTPA – 1:10; 1:20; 1:50, p-SCN-Bn-DOTA – 1:20) and 18 hours incubation on 4°C with gentle shaking. The binding is via thiourea linkage between amine groups of lysine residues of trastuzumab and isothiocyanato groups of chelators. The immunoconjugates were purified with six cycles of ultrafiltration and adjusted to concentration of 1 mg/ml. The trastuzumab conjugates were lyophilized to solid states with Labconco Free Zone Stoppering Tray Dryer.

After complete freeze drying the vials were closed and kept at 4°C for following examinations and for further labeling with radioisotopes (Lu-177 and Y-90).

Keywords: Trastuzumab, Immunoconjugates, Bifunctional chelators, Lyophilization, Radioisotopes.

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P-11

DNA METHYLATION ANALYSIS OF HIPPOCAMPAL TISSUE FROM AGED RATS

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Epigenetics, in particular DNA methylation, is an interesting field of research that differs fundamentally from conventional genetics. It describes an information transfer where the DNA sequence itself remains unchanged, but the base cytosine is modified. Methylation of cytosine is only observed if the base is located in front of a guanine within the DNA sequence (CpG). Multiple contiguous CpG sites (CpG islands) are often found in promoter regions, where methylation interferes with the binding of RNA polymerase, and therefore inhibits the transcription of the corresponding gene. The methylation process is reversible and known to be dependent on the environment and other conditions. In this study, aging is investigated as a possible epigenetic factor on memory formation in the hippocampus.

For this purpose three genes were chosen. Insulin-like growth factor 1 receptor (encoded by *IGF1R*) is an important component for cell survival and growth. Many studies have found a connection between insulin receptors and memory impairment. Silencing of the Fragile X Mental Retardation 1 gene (*FMR1*) causes the Fragile X syndrome (FXS), which is associated with mental retardation and autistic spectrum disorder. The corresponding protein (FMRP) has been found to show various posttranscriptional effects in neurogenesis. The protein encoded by Doublecortin X (*DCX*) is an important factor for dendrites in growing neurons and is often used as a marker for memory formation. It had been shown that the number of doublecortin mRNA decreases with aging and leads to memory impairment.

For this study, primers were designed for amplification of CpG-rich sites in the promoter region of the three genes in *Rattus norvegicus*. They were designed for an amplicon length of 80-130 bp containing as much CpGs as possible. In the following, PCR conditions were optimized and validated with methylated genomic rat DNA standards. After PCR, pyrosequencing was used to determine the methylation status of single CpGs.

The methods will be applied to hippocampal tissues from aged and young rats. In addition to DNA methylation status, gene expression on the mRNA level will be determined.

Keywords: DNA methylation, Pyrosequencing, Memory, Aging, Hippocampus.

P-12

IN VITRO EVALUATION OF FOOD-DICLOFENAC INTERACTIONS

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The absorption of diclofenac – a widely used nonsteroidal anti-inflammatory drug – can be influenced by the presence of different types of food. In this study the difference in membrane permeability of diclofenac in the presence of several types of foodstuffs (orange juice, milk, banana, etc.) was evaluated. For this purpose a modified Franz-cell, where the donor and receiving compartment was separated by an artificial membrane, was used. The amount of diclofenac in the receiving compartment was measured by an HPLC method with an UV-VIS detector at 276 nm. Beside the pure active ingredient, solid dispersions of diclofenac with PEG 6000 in 1:5 and 1:10 mass ratio were also evaluated in order to find a formulation of which the membrane transport is the least influenced by the presence of food. The results show, that the variances between the amounts of diclofenac in the receiving compartment, depending of the presence of foodstuffs, could be about fifty-fold. The tested solid dispersions show higher water-solubility, therefore the variability in the membrane transport of diclofenac was reduced using the solid dispersion of the active ingredient in the donor compartment. Based on these results the importance of studying food-drug interactions at pharmacokinetic level is concluded, primarily in the case of drugs which are indicated to be taken shortly after a meal.

Keywords: food-drug interaction, diclofenac, solid dispersion, membrane transport.

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P-13

**OPTIMIZATION AND VALIDATION OF CAPILLARY
ELECTROPHORESIS METHOD FOR SMALL-ANIONS
MEASUREMENT IN RED WINES**

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A capillary electrophoresis (CE) method has been developed and validated for analysis of organic acids (oxalate, tartrate, malate, malonate, pyruvate, succinate, acetate, citrate and lactate) and inorganic anions (sulfate and phosphate) in red wines. The separations were carried out in an automated separation system equipped with wide-bore (300 μm i.d.) fluoroplastic capillary with suppressed hydrodynamic and electroosmotic flow. Contact conductivity detection was used for monitoring of the separation and quantification of the analytes. Composition of carrier electrolyte was optimized to achieve sufficient resolution of organic and inorganic acids and adequate sensitivity of conductivity detection. The fast method (analysis time less than 5 min.) provided a good linearity of calibration curves ($R^2 > 0.992$) for the studied concentration range of the acids, as well as good reproducibility of migration times ($\text{RSD} < 1.5 \%$). The used fully automated separation system (sample dilution not included) predetermined the developed CE method for routine analysis. In total 33 red wines were analysed with the proposed method, including 22 Macedonian red wines from various varieties and geographic areas as well as 11 commercial red wines from different countries.

Keywords: Carboxylic acids, inorganic anions, capillary electrophoresis, conductivity detector, red wine.

Acknowledgement: The research was supported by the grants from SAIA (National Scholarship Programme of the Slovak Republic), VEGA 1/0342/15, APVV-0259-12 and CEEPUS RO-0010-1617 and Tikveš winery.

P-14

**DEVELOPMENT OF HPLC/DAD/MSⁿ METHOD FOR
AUTHENTICATION OF FRUIT PRODUCTS**

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Due to high costs of fruits, food companies may be tempted to increase their profit by adulteration. There are different possibilities how fruit products can be adulterated, e.g. by dilution, by blending with less expensive fruit species, by the addition of high fructose corn syrup or by the addition of artificial colours and aromatic compounds. For that reason it is very important to find different type of markers which indicate adulteration of different fruit products.

The goal of this study was to develop, optimize and validate methods allowing the authentication of various red fruits and products thereof. The focus of this study was on red fruits: blueberries (*Vaccinium myrtillus* and *Vaccinium corymbosum*), chokeberries (*Aronia melanocarpa*) and blackberries (*Rubus fruticosus*), seven pomegranate varieties (*Punica granatum*) and plums (*Prunus domestica*), because they are particularly rich with anthocyanins and other polyphenolic compounds. To achieve the goal HPLC/DAD/MSⁿ method was used for determination of polyphenolic fingerprints. The extraction procedure was performed with 80% methanol and 2 mM of NaF or ascorbic acid, using ultrasound for 30 min and then centrifugation for 15 min at 3000 rpm.

Principal component analysis (PCA) was then performed using the obtained results for the detected and quantified anthocyanins. From the results it can be concluded that delphinidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, cyanidine-3-glucoside, delphinidine-3-*O*-arabinoside, petunidin-3-*O*-galactoside and petunidin-3-*O*-glucoside can be used as the markers for blueberries, delphinidin-3-*O*-glucoside as marker for aronia, delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside as markers for pomegranates and cyanidin-3-*O*-glycoside-7-*O*-pentoside and peonidin-3-glycoside-pentoside as a marker for plums. The developed method was applied to five commercial juices from the market.

Keywords: adulteration, anthocyanins, berries, pomegranates, commercial juices, HPLC-DAD, MS.

Acknowledgement: Financial support provided by the Macedonian Ministry of Education and Science for the bilateral project between R. Macedonia and Austria titled: "Development of analytical methods for authentication of fruit products" is gratefully acknowledged.

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PINDOLOL – ENANTIOSEPARATION AND VALIDATION OF THE METHOD

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In pharmaceutical industry, drugs are often synthesized as a racemic mixture. Although all enantiomers of optical active compound have same physical-chemical properties, they can vary in their biological activity. In a case that some isomer provides undesirable side effect, it is important to separate it from mixture and check the purity of a drug.

Pindolol is an enantiomeric compound, belonging to the group of β -blockers, drugs which are blocking β -adrenergic receptors. However racemic mixture is used in clinical practice, R-(+) enantiomer has approximately 200x lower biological efficiency than S-(-) enantiomer [1] and thus decreasing the effectivity of this drug.

We are presenting new method for simultaneous enantioseparation and quantification of pindolol, using capillary electrophoretic system. This technique is simple and fast, with appropriate accuracy and precision with limits of detection and quantification comparable with those obtained by HPLC. Moreover this technique meets all rules of green chemistry.

Keywords: pindolol, capillary electrophoresis, method validation, enantioseparation.

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**RRLC-UV CHROMATOGRAPHIC METHOD USED FOR
DETERMINATION OF CHLOROGENIC ACID IN GREEN
COFFEE**

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Coffee is one of the most widely consumed beverages throughout the world due to its pleasant taste, aroma, stimulant effect and health benefits. It contains many bioactive compounds including chlorogenic acid which as a biologically active component plays significant role for antioxidant behavior of coffee. In this work was made an attempt to develop and validate a reverse phase rapid resolution liquid chromatography (RP-RRLC) method with diode-array detection (DAD) for identification and quantification of chlorogenic acid in green coffee. RP-RRLC analyses was performed on a Poroshell 120 EC-18 (50 mm x 3 mm; 2.7 μ m) column with acetonitrile/(water with 1 % phosphoric acid), (10/90, *V/V*) as a mobile phase. Flow rate of 1 mL/min was applied. UV detection was achieved at 325 nm wavelength. Chlorogenic acid was identified with comparison of the retention time of pure analytical standard with the retention time in analyzed samples. Taking into consideration that the new method was suitable for determination of chlorogenic acid in green coffee, further experiments were performed in order to determine linearity, sensitivity, selectivity, precision and accuracy. The linearity of developed method was in the concentration range from 12.33 to 143.50 μ g/mL with correlation coefficient values greater than 0.99. The recovery values were ranged between 97.87 and 106.67 % with the RSD values lower than 1 % suggesting excellent intra-day precision. The LOD and LOQ values under used chromatographic conditions were 0.29 and 0.96 pg, respectively. This method was successfully employed for quantitative determination of chlorogenic acid in five samples of green coffee. The developed method is simple, fast, sensitive and appropriate for the determination of chlorogenic acid in green coffee beans.

Keywords: chlorogenic acid, RP-RRLC, green coffee, extraction.

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P-17

**COUPLING CHROMATOGRAPHIC MICROFLUIDIC DEVICES
WITH GRAPHITE FURNACE ATOMIC ABSORPTION
SPECTROMETER**

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Microfluidics is one of the most dynamically developing field of the analytical chemistry. The need for a low-cost, compact, quick and efficient analytical procedure has been growing in many industrial applications, such as pharmaceutical, medical diagnostics, environmental and bioanalytical assays. The microfluidic devices provide great options to fulfill these demands. These chips are quite cheap to create and their use is environmental friendly due to the need of the small sample/eluent volumes and short analysis time [1]. Despite of the numerous advantages, there are only a very few microfluidic chromatographic systems applied with atomic spectrometry so far [2]. Although the use of graphite furnace atomic absorption spectrometry (GFAAS) allows us to work with a low detection limit applying a small amount of sample, no coupling of microchips with GFAAS was reported.

The aim of our work was to develop a coupled system, where we interface a C18 chromatographic microchip with a GFAAS for chromium speciation analysis. In our work we fabricated a poly(dimethylsiloxane) (PDMS) microchip, successfully separated Cr(III) and Cr(VI) on a micro column using ion-pair chromatographic method [3] and the chromium species were determined by GFAAS.

Keywords: graphite furnace atomic absorption, microfluidics, chromatography, chromium, speciation analysis.

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**APPLICATION OF Q-ICP-MS FOR SENSITIVE
DETERMINATION OF LEAD ISOTOPE RATIOS IN VARIOUS
ORGANICALLY BASED MATRIXES**

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Lead isotope measurements can provide useful information for routine means of “fingerprinting” the components grown in different habitats. If lead is present in the soil, a plant will take up small amounts and subsequent isotope ratio studies might provide unique means of differentiating between different plant sources of origin. Studies of the isotopic composition of lead are therefore commonly used in the environmental science as well as geological and anthropological studies. Among all the naturally occurring lead isotopes, only ²⁰⁴Pb is non-radiogenic, whereas, ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb are the daughter products from the radioactive decay of ²³⁸U and ²³⁵U and ²³²Th, respectively. As a consequence, small Pb isotope abundance variation occurs in nature and the isotopic composition of lead in the environment is dependent on local pollution sources. Being able to accurately measure all of the Pb isotopes is important for a number of investigations. Quadropole inductively coupled plasma with mass spectrometry (Q-ICP-MS) was used to investigate whether this chemical application can offer a reliable and practical solution to the problem of the polyatomic overlap in the presence of organically based matrix samples. Very good sensitivity was obtained for ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb isotopes, while, ²⁰⁴Pb suffers from isobaric interference from ²⁰⁴Hg. Satisfactory linearity (R) was obtained in the range from 5 to 100 µg/L. The study summarizes the instrument optimization procedure for Pb isotope measurements in wine and various plant samples. The calculated isotope ratios ranges: a) ²⁰⁷Pb/²⁰⁶Pb: from 0.985 to 1.122 with standard deviation of data distribution of 0.038 and b) ²⁰⁸Pb/²⁰⁶Pb: from 2.221 to 2.998 with standard deviation of data distribution 0.21. Also, the isotopic ratios were presented for samples from same and from different geographical region.

Keywords: lead, isotope ratio, wine, plant species, Q-ICP-MS.

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**ASSESSING THE BIOAVAILABILITY AND TRANSLOCATION
EFFICIENCY OF MINERAL ELEMENTS IN *Lycium barbarum*
SPECIES FROM R. MACEDONIA AND R. CHINA**

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Goji berries or wolfberries (*Lycium barbarum* L.) have been traditionally used as food and a medicinal plant. The interest in the chemical composition of goji berries has intensified because of an increased awareness of their possible health benefits. On the other hand, the toxic elements can also be interacting in the plant tissue through the bioavailable pathway of the root-soil system. The present work reports the results obtained by the proposed method for the simultaneous determination of metals (Na, Mg, K, Ca, Mn, Fe, Cu and Zn) and nonmetals (P and S) in goji berries by using inductively coupled plasma–optical emission spectrometry (ICP-OES), following digestion using a diluted oxidant mixture in a closed-vessel microwave oven. Determinations of Cr, As, Pb, Cd and Ni were realized using the graphite furnace atomic absorption spectrometer (GFAAS). Mercury quantification was realized on the solid samples by the TDAAS method, with the automated direct mercury analyzer hydra-C. The obtained data for element contents reports the multi-element characterization of different plant parts, and variation in multi-elements content between Macedonian and Chinese species. Bioaccumulation and bioconcentration factor scores revealed the translocation efficiency of metals and nonmetals across the *Lycium barbarum* plant parts.

Keywords: *Lycium barbarum*, multi-elements content, ICP-OES, GFAAS, TDAAS.

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CHEMICAL PROPERTIES OF CERTAIN VARIETIES OF TOBACCO

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Chemical properties of raw tobacco is an important indicator of its value in use, depending on the presence of certain chemical components and their relationship. Chemical properties largely depend on agro ecological conditions, applied agro-techniques, insertion, time of harvest in technological maturity of the leaf etc. Chemical properties, in a synthesis with the technological and degustative properties of the raw material are relevant indicator for determining the quality of tobacco. When analyzing the results of chemical testing in this research, the quality of tobacco was determined by Schmuck number (coefficient between the soluble carbohydrates and protein content). The experiment was set in a randomized block system in four repetitions on two oriental tobacco cultivars (*PNS72* and *YV 125/3*), each planted in three variants: variant 1-control (conventional production of seedlings); variant 2-N and variant 3-P (Floating Tray System production). Tobacco material was prepared by an average sample (soft ground tobacco powder), each variety and variant of industrial class unik1-3. Determination of chemical composition of the tobacco was performed according to standard methods of analysis. The following chemical components were tested: nicotine, proteins, total nitrogen, ash and pH of tobacco. Statistical data of the processing method was used. The analysis of variance and differences were tested by F-test and LSD-test (SPSS for Windows, procedure Sum of squares, Model III). The index of participation of the chemical components versus control was performed. The best results were achieved from tobacco raw material obtained from FTS production of seedling (N and P) for both cultivars (lower nicotine content, total nitrogen and proteins and higher content of soluble carbohydrates). The results demonstrated that the ratio of quality expressed through Schmuck quality index was higher in cultivar *YV 125/3* in terms of cultivar *PNS72*. Schmuck quality index showed a positive value (greater than one) which implied high quality of oriental tobacco.

Keywords: oriental tobacco, chemical properties, Floating Tray System.

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P-21

**PHOSPHORUS AND POTASSIUM AVAILABILITY IN SOILS
USED FOR TOBACCO CULTIVATION IN THE REPUBLIC OF
MACEDONIA**

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Macronutrients and their availability are crucial to plants but also their excessive contents in soil are very harmful. Tobacco production and land management in the Republic of Macedonia has a long history; therefore, P and K content in soils are impacted not only by soil parent material, but also by anthropogenic activities. The main purpose of this study was determining spatial distribution and impact factors influencing the P and K availability that will help in applying certain measures for agricultural and environmental management. Soil properties (pH, cation exchange capacity, organic matter), available (DTPA extracted) Fe, Mn, Cu, Zn content and total and available P and K were analyzed on 150 topsoil samples (0-30 cm) from different agricultural areas. Pearson's correlation coefficients were calculated for each variable to reveal the relationships between the availability ratios (available content/total content) of these two macronutrients and the selected soil properties. Results show that availability ratio of every macronutrient is negatively correlated with its total content and positively correlated with its available content, implying that the total K and P content in soil is relatively stable over the study area and a considerable available amount comes from outside input. P availability ratio is mainly controlled by available and total P, pH and available Fe. K availability ratio is mainly affected by pH. Due to the heterogeneous land use and management, availability ratios of K have larger variation than that of P. However, proper adjustment of macronutrient content in some study sites is needed.

Keywords: macronutrients; oriental tobacco; availability.

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P-22

**UV SPECTROSCOPY METHOD USED IN DETERMINATION
OF DISSOCIATION CONSTANT OF SOME *p*-NITRO-*p*-
SUBSTITUTED BENZOILHYDRAZONES**

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Dissociation constants are most valuable parameters to understand chemical phenomenon such as biological activity, absorption and extent of ionization of organic compounds in different pH. There are various methods for determination of dissociation constants among which the ultraviolet-visible spectrophotometry is one of the most frequently employed. The importance and wide application of hydrazones is due to biological activity that they possess which generally depends on the pH values of the media. The objective of this investigation was to determine thermodynamic dissociation constants some *p*-nitro-*p*-substituted benzoilhydrazones in sodium hydroxide media applying UV-Vis spectroscopic method. In neutral medium two absorption bands located at 195 nm and 330 nm were noticed in the UV spectra of investigated hydrazones. The bathochromic shift of the second absorption band was observed increasing the pH of the solution. The changes in the spectra indicated that dissociation process takes place in one step, except for hydrazone with phenolic group in its molecule. The pH region of dissociation for the first step ranges between 10.8 and 11.6, while for the second step ranges between 11.7 and 12.1. Using the changes in the UV spectra which appear as a result of the dissociation process the dissociation constants were determined numerically and graphically. Dissociation constants were calculated according to equation $\text{pH} = \text{p}K_{\text{HA}} + \log I/(\text{dissociated drug/neutral drug})$ for weak acids, while graphically were obtained as an intercept of the dependence of $\log I$ on pH. In order to determine the thermodynamic dissociation constants measurements were performed at ionic strength of 0.1, 0.25 and 0.5 mol/dm³ (NaClO₄). There was a good agreement between the obtained $\text{p}K_{\text{HA}}$ values of the investigated hydrazones and those of similar classes of compounds.

Keywords *p*-substituted aromatic hydrazones, dissociation, UV spectrophotometry, dissociation constants.

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**STUDY OF THE RELATIONSHIPS BETWEEN THE
STRUCTURE AND BIOLOGICAL ACTIVITY OF SOME
SUBSTITUTED AROMATIC HYDRAZONES**

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Hydrazone derivatives represent one of the most active classes of compounds possessing a broad spectrum of biological activity. The use of the hydrazones in medicine is due to their antiinflammatory, antimicrobial, antidepressant, antitumoral, analgesic, antiplatelet, antischistosomiasis, anticonvulsant and antiviral activity. Due to their physiological activity, they are used as herbicides, insecticides, and plant growth stimulants. QSAR analysis of a series of previously synthesized *p*-substituted aromatic hydrazones tested for growth inhibitory activity against *Bacillus subtilis*, was performed using several physicochemical descriptors: Surface tension (ST), Molar Refraction (MR), Molar Volume (MV), Parachor (Pc), Index of Refractivity (η), Density (D) and Polarizability (α). One, two-parameter and three models were obtained and validated by using several statistical parameters: R ; R^2_{adj} ; F-test; Sd; R_{ped} ; PRESS/SSY; Q^2 ; S_{PRESS} ; PSE and Q. Both the parameters (D and α) contributing to statistically best model (two-parametric model: $R^2 = 0.9857$; Sd = 0.0049; $R^2_{adj} = 0.9833$; F-test = 412.298; $R_{pre}^2 = 0.985$; Q = 202.6172; PRESS/SSY = 0.0158; $S_{press} = 0.0050$; PSE = 0.0045; $Q^2 = 0.9842$) have positive input to the modeling of biological activity of selected hydrazones.

Keywords: substituted hydrazones, QSAR, descriptors, statistical parameters.

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SYNTHESIS OF NOVEL AMINOMETHYL DERIVATES OF QUINOLONES

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The new era in treating bacterial infections started with the discovery of quinolone compounds. They have demonstrated broad spectrum of applicability against many bacterial species as antibiotics, but unfortunately their uncontrolled administration led to the development of bacterial resistivity. Many efforts are made in order to synthesize new analog compounds hoping that they will manifest more efficient antibacterial properties. The focus of this research is synthesis of aminomethyl derivatives of some quinolones such as norfloxacin, ciprofloxacin and pipemidic acid and comparison of their antibacterial properties. The synthesis of these derivatives is achieved in two steps. In the first step, a Mannich base of norfloxacin, ciprofloxacin and pipemidic acid are synthesized, while phthalimide is used as nucleophile. Thereby, in the second step, the obtained phthalimidomethyl derivatives are hydrolyzed with hydrazine hydrate to finally produce the aminomethyl derivatives. Each derivate is characterized with primary amino group that behaves as a nucleophile, which can participate in many reactions, leading to synthesis of new compounds. The qualitative analysis of the precursors and obtained products is performed using FTIR and melting point determination. In our further microbiological experiments, the comparison of the antibacterial activity will show which of the synthesized derivatives will achieve the best antibacterial activity vs. norfloxacin, ciprofloxacin and pipemidic acid, expressed as minimum inhibitory concentration and zone of inhibition.

Keywords: Quinolones, Gabriel synthesis, Mannich bases, Antibacterial activity.

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P-25

**PREPARATION OF SILVER NANOPARTICLES USING
ASCORBIC ACID AND GLUTATHIONE AS REDUCTIVE
REDOX AGENTS**

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Silver nanoparticles have been formed with two different methods: by reduction of Ag^+ ions from AgNO_3 aqueous solution and from Ag electrode (with electrolysis). Glutathione and ascorbic acid have been used as reductive redox agents in acidic (ammonium acetate/acetic acid buffer at pH 4.8) and in neutral medium. The preparation has been conducted by mixing equimolar solutions of reactants at concentrations of 10^{-4} and 10^{-6} mol/dm³. Formation of colloidal solutions containing silver nanoparticles has been confirmed by electrochemical, spectroscopic and microscopic techniques. The declining of the concentration of the free Ag^+ ions following the reduction with the reductants has been measured by anodic stripping voltammetry using square-wave voltammetry as a potential modulation form. By applying UV-Vis spectroscopy in solutions with concentration of 10^{-4} mol/dm³, the formation of nanoparticles has been supported with the specific surface plasmon resonance absorption peak at 350 nm. Finally, the morphology and dimensions of the formed silver nanoparticles have been studied by inspecting microphotographs collected by atomic force microscopy. Considering morphology, uniformity and dimensions of the formed nanoparticles the best results have been obtained by applying glutathione as a reductive agent, most likely due to its ability for both complexation and reduction, as well as due to the slow kinetics of the redox reaction.

Keywords: Silver nanoparticles, SWV, UV-Vis spectrometry, AFM, ammonium acetate/acetic acid.

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**RELATION BETWEEN STRUCTURE AND ACTIVITY OF
ANTISEPTICS AND DISINFECTANTS THAT ARE USED IN
CLINICS**

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The aim of this study was to review the usage of antiseptics and disinfectants in selected hospitals in Strumica, Ohrid, Veles, Stip, Kavadarci and Gevgelija and their usage as a suitable way for prevention of interhospital infections, which can cause serious problems in the modern medicine. The word intrahospital or nosocomial means infections that develop in hospitals or are caused by microorganisms acquired in time of the hospitalization of the sick and their clinical manifestations occur 48–72 hours the earliest from the day that the hospitalization occurred. To achieve the purpose of lowering and prevention of these infections, number of precautions and procedures are taken into practice routinely in the hospitals. The data from the annual reports for antiseptics and disinfectants such as: Bactosal, Ecosal, Dezintal, Betadine, Hydrogen peroxide, Formaldehyde and Ethanol, used on the selected departments for gynecology, surgery and transfusion, were collected. Our purpose was to find correlation between the structure of the antiseptics and disinfectants and the range of their activity (bacteriostatic or bactericidal). Despite of their structure it seems that physical and chemical properties of the solutions used as antiseptics and disinfectants are important for their activity.

Keywords: Antiseptics, Disinfectants, Intrahospital infections, bactericidal, bacteriostatic.

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**FAST DETERMINATION OF GLYPHOSATE IN BEER BY
ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-
TRIPLE QUADRUPOLE MASS SPECTROMETRY**

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Glyphosate is widely used herbicides, and has been considered nontoxic posing with minimal risk to human health presence of a persistent exposure at trace levels. However, recent toxicity evaluations by the World Health Organization's (WHO) International Agency for Research on Cancer classified it as “probably carcinogenic to humans”. Their ionic, water-soluble and high polarity characteristics of glyphosate make this compound very difficult for analysis. A simple and fast method for the determination of glyphosate in beer based on ultra-performance liquid chromatography (UPLC) coupled to triple quadrupole mass spectrometer (TQ/MS), using PFP chromatography column was developed and validated. The optimal quantification and confirmation transitions, their respective fragmentation, collision energies, cell accelerator voltage, polarity and retention time with delta retention time windows were optimized using Mass Hunter Optimization Software. Sample preparation consisted in just dilution of the beer sample with 0,1 % HCOOH to minimize the matrix signal suppression effects. Method performance was evaluated during validation through a series of assessments that included linearity (in the range of 10 - 1000 µg/L) with (R^2) more then 0.99; accuracy (expressed as a recovery on three different levels in the range of 82,0 – 92,5 %); precision (expressed as a $RSD < 2$ %); limits of quantitation (LOQ) were determined to be 10 µg/L (ppb). The validated method was applied to detect and quantify glyphosate in Macedonian beers.

Keywords: glyphosate; beer; validation; PFP column, UPLC-TQ/MS.

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**IDENTIFICATION OF PESTICIDES IN GROUNDWATER
SITUATED UNDER GREENHOUSE AGRICULTURE
PRODUCTION AND DROPPING IRRIGATION, USING GC/MS
PULSED SPLITLESS INJECTION**

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The aim of this study was to investigate the quality of groundwater situated under a topsoil where greenhouses and dropping irrigation system are used in tomato production. A GC-MS method was applied using pulsed splitless injection with pressure of 50 psi and purge flow to split vent of 1.5 minutes [1], for the screening of the most frequently used pesticides such as benalaxyl, chlorpyrifos, malathion, pirimifos methyl, methomyl, metribuzin, penconazole, triadimenol, pyrimethanil, and buprofezin. Seventy eight groundwater samples were collected from the region of Strumica, an agriculturally vulnerable area regarding pesticide application, during 2014 - 2015. Slightly modified liquid-liquid extraction was performed using dichloromethane as a solvent [2]. The obtained results show negligible pollution of groundwater with investigated pesticides. Only 5% of investigated samples were polluted with pesticides in concentrations which doesn't exceed the national maximum concentration limit. The analysis showed the presence of pyrimethanil and chlorpyrifos in maximum concentrations of $0,0299 \pm 0,00026 \mu\text{g/l}$ and $0,133 \pm 0,00929 \mu\text{g/l}$, respectively. The main reason for this negligible pollution of groundwater with pesticides is considered to be the use of greenhouses and the dropping irrigation system in agriculture production which doesn't allow high quantity of water to be able to pass from the soil surface downward to the aquifers.

Keywords: GC/MS, pulsed splitless injection, pesticides, groundwater, dropping irrigation.

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**DEVELOPMENT OF A SINGLE-DROP MICROEXTRACTION
METHOD FOR GC-MS ANALYSIS OF BISPHENOL A IN
WATER SOLUBLE MATRICES**

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Single-drop microextraction (SDME) is one of the simplest and very easily implement techniques for extraction of analytes from complex matrices. It is very inexpensive since it is usually performed in a GC syringe and environmentally friendly since only a drop of a solvent is used. However, there are issues of reproducibility and applicability due to the nature of the technique [1].

In order to test this technique we have attempted to develop a single-drop microextraction followed by in-syringe derivatization and GS-MS determination for the analysis of bisphenol A in water samples. 3 mL water samples spiked with various concentrations of bisphenol A were extracted with a single 3 µL drop of hexane and the conditions such as extraction temperature and time were optimized. The extraction was followed by derivatization with [*N,O*-bis (trimethylsilyl) trifluoroacetamide] inside the syringe barrel. Various temperatures and times of derivatization were tested in order to obtain the best yield of the desired product. The developed method was applied to water samples of commercial bottled waters.

Keywords: single-drop microextraction; bisphenol A; GC-MS; sample preparation, water analysis.

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**SOYBEAN VARIETIES AS EFFECTIVE TOOL FOR
PHYTOREMEDIATION OF CADMIUM POLLUTED SOIL**

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Cadmium (Cd) is an important toxic heavy metal and the warning of health risks from Cd pollution were issued initially in the '70s. Increased concentrations of Cd in agricultural soils are known to come from human activities, such as the application of phosphate fertilizer, sewage sludge, wastewater, pesticides, mining and smelting of metalliferous ores with high Cd content, and traffic. Although there are many reports on Cd contamination in agricultural soils, most of the investigations are concentrated in the vicinity of the mine sites. But concerning point is certainly dealing with the problem of cadmium soil pollution. Remediation has been improved as most effective for soil pollution. Recently phytoremediation has been improved as one of the most convenient techniques for remediation of heavy metals from contaminated soils. The main purpose of the present study was to determine the effectiveness of soybean varieties for phytoremediation of agricultural soils with higher content of cadmium. For that purpose, three soybean varieties with long vegetation (*Balkan*, *Ilindenka* and *Pavlikeni*) and soybean varieties with short vegetation (*Pella*, *Avigea* and *OW*) were used in association with rhizobacterium *Bradyrhizobium japonicum*. The total and available content of cadmium were determined in separate parts of the plant (root, stem, leaf, seed and pod). Physicochemical analyses were conducted for determination soil properties. Bioaccumulation factor (BAF), bioconcentrating factor (BCF) and translocation factor (TF) were used to examine the soybean potential for cadmium remediation.

Keywords: cadmium, soybean varieties, phytoremediation, soil pollution.

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**ASSESSMENT OF ARSENIC POLLUTED GROUNDWATER IN
THE STRUMICA REGION, AN INTENSIVE AGRICULTURE
PRODUCTION AREA**

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Arsenic polluted groundwater was found in the south-east part of the Republic of Macedonia where an intensive agriculture production is concentrate on the area of 963 km². Out of 185 samples collected from boreholes, 64 samples have arsenic in concentrations greater than 10 µg/L, from which 30 samples have concentration greater than 50 µg/L with maximum concentration of 176.56 µg/L. The affected aquifers are mostly concentrated in the central part of the valley characterized with alluvial plains and young aquifers. Polluted samples are collected from boreholes with different depths: 15 samples are shallow (4,5 - 20 m), 42 samples are deep (21-100 m) and 7 samples have depth greater than 100 m. The contaminated groundwater is slightly acidic to neutral (pH between 7.5 – 8.53), with high alkalinity (HCO₃⁻ 177.06 – 511.87) and moderate conductivity (EC_w 2.48 – 7.2). Highly affected samples are characterized with high concentrations of Mn and Fe. Other investigated ions such as Mg, Na, K, Ca, P, Cu, Ni, Co, Zn and Pb are present in low concentrations. Factor analysis revealed high positive correlation between arsenic, iron and manganese which suggest the natural origin of arsenic in groundwater. Reducing environment, high iron, high manganese and bicarbonate content, as well as low sulfate and nitrate content, show that reductive dissolution is one of the mechanisms by which arsenic is released into the groundwater [1].

Keywords: agriculture, boreholes, irrigation, geochemical composition, factor analysis.

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**MACRO AND TRACE ELEMENTS BIOAVAILABILITY IN
VEGETABLE AND HERBAL SPECIES FROM POLLUTED AND
CONTROL AREAS**

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Several plant species (*R. acetosa*, *S. oleracea*, *U. dioica*, *A. Sativum*, *A. cepa* and *P. hortense*) were studied to determine macro and trace elements content trends and their bioavailable forms. The total contents of 21 elements: (1) macro biogenic elements (Ca, K, Na, Mg, and P), (2) elements that have the essential functionality in microcontents (Ba, Cr, Li, Cu, Fe, Mo, Mn, Sr and Zn), and elements that are toxic even in traces (Ag, Al, As, Cd, Ni, Pb and V) were determined with the application of atomic emission spectrometry with inductively coupled plasma (ICP-AES). The total concentrations were measured in samples after total mineralization with concentrated nitric acid and hydrogen peroxide in a microwave digestion system. Three extraction methods were implemented for determination of bioavailable contents in the soil: (1) Extraction with deionized H₂O that provides information on the actual availability of the elements in the soil solution; (2) Extraction with 0.1 M HCl. (3) Extraction of the soluble species of trace elements in a mixed buffered solution (pH= 7.3) of triethanolamine (TEA, 0.1 mol L⁻¹) with CaCl₂ (0.01 mol L⁻¹) and diethylenetriaminepentaacetic acid (DTPA, 0.005 mol L⁻¹), which is often recommended for extraction of toxic or biogenic metals. Translocation enrichments were obtained for As, Cd, Cu, Ni, Pb and Zn. All of the analyzed species show potential for phytoextraction and phytostabilization of Cd, Cu, Pb and Zn.

Keywords: vegetable; herb; heavy metals; bioavailability; ICP-AES; multivariate.

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**MULTI-ELEMENT CONTENT CHARACTERIZATION OF
COLD PRESS EDDIBLE OILS PRODUCED FROM TWELVE
SUNFLOWER VARIETIES**

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The content of the following isotopes of the 36 elements (Li⁷, Be⁹, B¹¹, Na²³, Mg²⁴, Al²⁷, P³¹, Ca³⁹, Ti⁴⁸, V⁵¹, Cr⁵³, Mn⁵⁵, Fe⁵⁶, Co⁵⁹, Ni⁶⁰, Cu⁶³, Zn⁶⁴, Ga⁷¹, Ge⁷⁴, As⁷⁵, Se⁷⁷, Rb⁸⁵, Sr⁸⁸, Mo⁹⁵, Pd¹⁰⁶, Ag¹⁰⁷, Cd¹¹¹, In¹¹⁵, Sn¹²⁰, Sb¹²¹, Cs¹³³, Ba¹³⁷, Tl²⁰⁵, Pb^{206/207/208} and Bi²⁰⁹) in edible oils produced from twelve sunflower varieties from Republic of Macedonia were determined, using inductively-coupled plasma-mass spectrometry (ICP-MS) after microwave digestion, employing nitric acid and hydrogen peroxide in this step. The method has been validated using both an oil reference material and recovery experiments over different oil samples, obtaining satisfactory results in both cases. Interday repeatability lower than 10% was observed for all of the analyzed elements in the analyzed oil samples. Studying the multi-elements content, in order to detect tendencies in the oil samples between varieties, principal components analysis was used. Promising groupings were observed using a model with two principal components and retaining 82.3% of the variance.

Keywords: edible oil; sunflower varieties; multi-element content; bioavailability; ICP-MS; multivariate analysis.

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METAL ION MEDIATED MOLECULAR IMPRINTED POLYMERS FOR ATENOLOL

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Molecular imprinting allows the design of highly cross-linked polymeric materials able to mimic natural recognition processes. The choice of the appropriate functional monomer, cross-linker and the nature and specificity of template - monomer interactions are critical for a successful imprinting process since creating recognition sites with chemical and steric complementarity are prerequisites for high selectivity and permanent memory towards the imprinted species. Use of a metal ion (pivot) that mediates the interaction between the monomer and template (ligands) has proven to offer a high fidelity of imprint by inducing a higher degree of organization in the prepolymerization mixture due to the spatial oriented and superior stability of the metal-ligand bonds.

The pivoting effect of two transition metal ions (Cu(II), Co(II)) has been tested in different porogenic solvents (DMF, ACN, MeOH) using (S)-atenolol as template. The bulk molecular imprinted polymers (MIPs) were obtained by photoinitiation at low temperature -18°C using N,O-bismethacryloyl ethanolamine (NOBE) as single cross-linking monomer. For comparative reasons atenolol imprinted methacrylic acid and trifluoromethacrylic acid based MIPs were also synthesized. Upon bulk synthesis, the polymers were ground and sieved, and the resulting particle slurry (25-35 µm average diameter) was filled in 100 x 2.1 mm HPLC columns. The chiral selectivity of the adsorbents was assessed based on the recorded selectivity factors for atenolol's enantiomers in different chromatographic conditions using a binary mobile phase (ACN with 100 mM phosphate buffer (pH=3) and 50 mM borate buffer (pH=9), respectively) in isocratic elution mode.

Results showed that in spite of the expected favorable non-covalent interaction between the template molecule and NOBE, acrylate based MIPs demonstrated superior enantioselectivity under the tested conditions. Moreover, the studied metal pivots showed no significant improvement in enantioselectivity.

Keywords: metal ions, metal pivot, atenolol, molecular imprinting, molecular imprinted polymers.

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**MOLECULARLY IMPRINTED POLYMER BASED
ELECTROCHEMICAL SENSOR FOR THE TRACE ANALYSIS
OF CLARITHROMYCIN**

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Antibiotic resistance has a significant impact on health and economy, WHO recommending an urgent improvement in the surveillance of the use of antibiotics, hence the need for developing new analytical sensors, capable to detect selectively low concentrations of antibiotics from different matrices.

The purpose of this study was to develop an electrochemical sensor for the detection of clarithromycin from environmental and pharmaceutical samples, a widely used macrolide antibiotic. A highly selective interface is essential in creating electrochemical sensors capable of detecting the target molecule in a complex matrix, even in the presence of closely-related structural analogues. Molecular imprinting enables the synthesis of robust polymers with tailored selectivity towards the target molecule. Covalently imprinted polymers present a high selectivity based on double recognition mechanism, both through functional and spatial complementarity.

Therefore, molecularly imprinted polymers (MIPs) based on several boronic acid functional monomers (cis-propenylboronic acid, trans-3-phenyl-1-propen-1-ylboronic acid, (1E)-(pent-1-en-1-yl)boronic acid, 3-thienyl boronic acid) were tested, capable of forming stable cyclic esters through covalent interactions with the cis-diol moieties of clarithromycin. The polymeric films were electrosynthesized by cyclic voltammetry on the electrode surface by copolymerizing the functional monomer with the cross-linker (pentaerythritol triacrylate or 2,2'-bithiophene).

Indirect electrochemical analysis of clarithromycin was performed using a redox probe ($[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$). The selectivity of the MIP sensor towards clarithromycin was demonstrated by comparing with bare and nonimprinted polymer electrodes.

Keywords: electrochemical sensors, antibiotics, clarithromycin, molecular imprinting, molecular imprinted polymers.

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**SCREENING OF POTENTIALLY ACTIVE COMPOUNDS FOR
DM1 TREATMENT BY AFFINITY CAPILLARY
ELECTROPHORESIS**

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Myotonic dystrophy type 1 (DM1) is a trinucleotide repeat disorder characterized by the abnormal expansion of a CTG triplet in one of the non-coding regions of the DMPK (dystrophia myotonica protein kinase) gene. The main mechanism of the disease is the transcription of the CTG triplet into RNA (CUG)_n forming hairpin structures, capable of binding different proteins and small molecules in the cell [1]. The toxicity of the RNA triplet itself is manifested by two means, either by binding proteins necessary for the alternative splicing of pre-mRNAs or by accumulating in the nucleus. Unfortunately for the moment there are no clinical validated treatments for DM1, but several theoretical approaches are being investigated. These are trying to diminish the toxicity of the RNA triplet by using small molecules or antisense oligonucleotides to destroy or to inactivate it [2,3]. In this work we use affinity capillary electrophoresis as screening method for novel potentially active compounds for the treatment of DM1. For these purpose we employed several DNA and RNA probes, corresponding to both normal and pathological models. For the development of the ACE method we used pentamidine as leading compound, but the end the ligands tested were more numerous. The results obtained in our work allowed us to estimate some binding parameters like the affinity constant or the stoichiometry. This data could improve the workflow of ligand screening for DM1, saving time and reagents due to the inherent features of capillary electrophoretic techniques.

Keywords: Myotonic dystrophy type 1, alternative splicing, triplet repeats expansion, nucleic acids ligands, affinity capillary electrophoresis.

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**ELECTROPHORETIC ANALYSIS OF HAIR PROTEINS
ISOLATED BY SIMPLE EXTRACTION METHOD**

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Human hair is a filamentous biomaterial which primarily consists of proteins. We developed a rapid and convenient extraction procedure for total hair proteins to examine the influence of bleaching. This procedure is based upon the fact that the combination of denaturing agents such as thiourea and urea in alkaline solutions of Tris buffer containing reducing agent – mercaptoethanol, can effectively remove proteins from the cortex part of human hair.

Extracted hair proteins have been analyzed by gradient sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Two main protein fractions of microfibrillar keratins and keratin-associated proteins (9-33 kDa) have been detected. Keratin fractions type I (43-44 kDa) and type II (55 kDa) have been determined by electropherograms processed by GelPro v31 of chemically untreated hair extracts and 72% of total proteins were found. Applying this method on bleached hair with 9 % H₂O₂, a significant decrease in the amount of each protein fraction was found, but changes have been mostly observed on keratins type I and type II.

This method is available for studying the genetic variations and post-translation modification in the matured keratinized tissues and also for application in the textile and cosmetic industry.

Keywords: Proteins, Hair, Keratin, KAP, SDS-PAGE

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